EXPERIMENTAL EMBRYOLOGY

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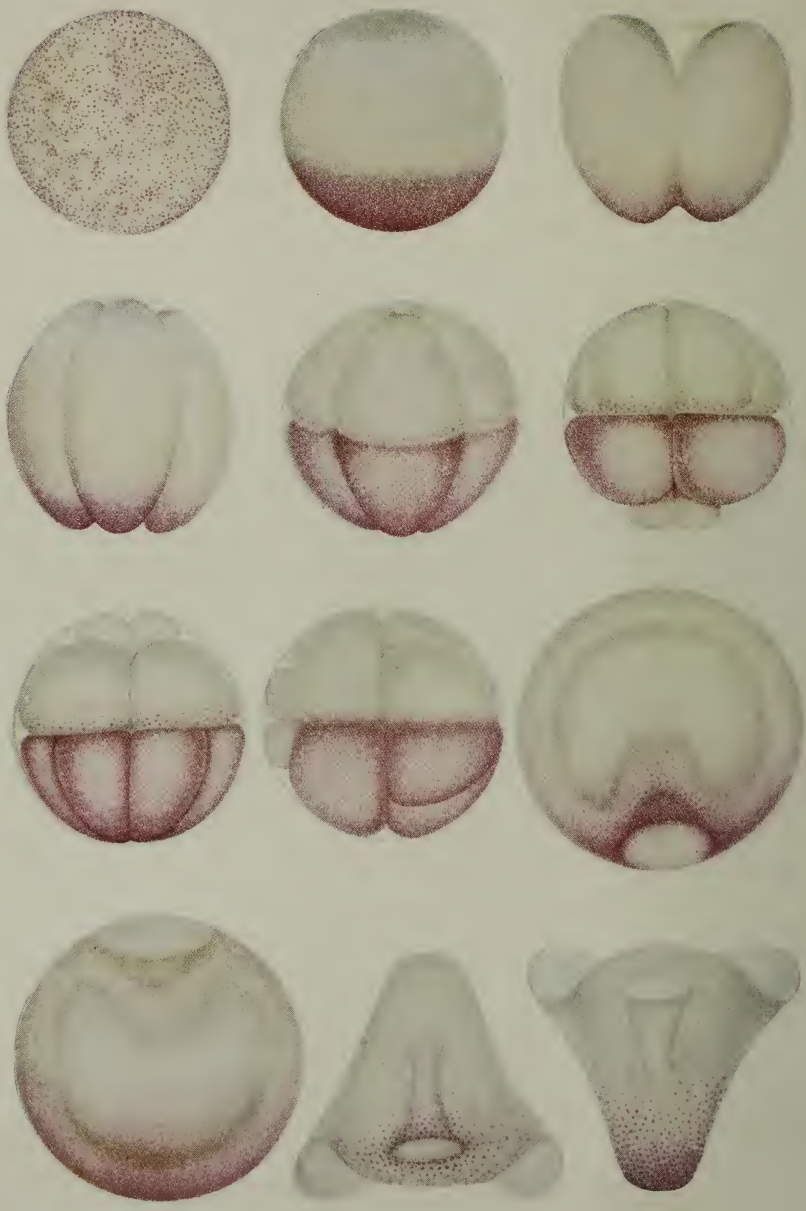

Development of the Centrifuged Eggs of Arbacia

EXPERIMENTAL EMBRYOLOGY

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PREFACE

Between the years 1897, when I brought together the somewhat meagre results of experimental embryology in my book on "The Development of the Frog's Egg: An Introduction to Experimental Embryology," and the present time, 1927, a very extensive literature has grown up covering a wide field of experimental research in embryology. Those who have followed this growth realize that many changes in outlook have taken place. The analysis of the first-found results of the experimental study of development of the egg, called Developmental Mechanics, soon became entangled in futile philosophical discussions about vitalism *versus* mechanism. Some discouragement and relapse was inevitable, but nevertheless a small band of individuals kept at work inventing newer and better methods of objective study, and trying out new experimental tests. There followed a period in which more attention was given to the study of embryological problems from a purely physiological standpoint and by chemical methods. This seemed to be, and was, an improvement, but was compromised to some extent by ignoring the facts then known, showing that the structure of the egg cannot be disregarded if its development, rather than its chemical composition, is the goal to be sought. Nevertheless, the study of the chemical field has made many valuable additions to our knowledge. Meanwhile the properties of colloids were being investigated, and the realization that protoplasm contains materials in the colloidal state pointed to new possibilities unsuspected before by embryologists. Other openings appeared, notably the possibility of treating the developmental process as a system whose reactions could be measured by physical standards, and interpreted in terms of physical constants.

In attempting to give an account of present day experimental embryology which is emerging from the pioneer stage and approaching more nearly an experimental science, there is a serious

difficulty in presenting the subject in its true historical setting. First of all there is the ever-present temptation to make undue use of the most recent and best illustrated contributions. This cannot fail to leave the impression that the most significant work is the latest and most detailed, and the original discoveries that led to these later expansions may fail to receive the proper credit. The beginnings often go back to relatively brief and incomplete contributions that contain a really original idea, or to the introduction of a new method of research. New ideas that open up new lines of research are rare, and new methods of research that are fruitful are also rare, while the application of a new idea or method after it has been suggested is a relatively simple and common procedure, especially in a subject as rich in material and as unexplored as is embryology. Familiarity at first hand with the earlier or first contributions will sometimes show that the suggestion or discovery has not come out of a clear sky, but rests in turn on other work that has preceded it. Chance, too, rather than foresight, plays its rôle. Again, it is to be remembered that novel ideas are sometimes at first mere guesses. Most of them disappear; a few survive—not always because they had at first more to support them, but because later work showed that they were in the direction of further advance. Hence, any attempt to assign relative values to the earlier observations is an almost impossible task. Yet an attempt to put these discoveries in their historical setting must be made, even although it is obvious that we are still too near to the work to see it in its true perspective.

In the present volume many of the problems are concerned with the first changes and movements in the cytoplasm prior to its cleavage, and with the movements of groups of cells following cleavage—in a word, with the so-called formative changes in the egg and with their initiation. It is my intention to consider the more obviously physiological changes in another volume, where such topics will be discussed as growth, reflex reactions and tropistic movements of larvae, sex-determination, embryonic grafting, the influence of the environment on the development of the embryo, the source of the energy of development, etc. The incompleteness of some of the topics in the present volume will then be supplemented, and a more rounded treatment of the present condition of experimental embryology will be attempted.

A transparent egg as it develops is one of the most fascinating objects in the world of living beings. The continuous change in form that takes place from hour to hour puzzles us by its very simplicity. The geometric patterns that present themselves at every turn invite mathematical analyses. The constancy and orderliness of the whole series of events, repeating themselves a thousandfold in every batch of eggs, assures us of a causal sequence conspiring to create an object whose parts are adjusted to make a machine of extraordinary complexity.

This pageant makes an irresistible appeal to the emotional and artistic sides of our nature. Hence not without a feeling of jealous regret, the old-fashioned embryologist sees these gems of nature consigned to test tubes for chemical analyses, to centrifuges to disturb their arrangements, to microdissecting instruments to pick them to pieces, and to endless tortures by alterations in the environment to disturb the orderly, normal course of events. For, it is the automatic self-contained perfection of the developmental process that holds our interest. Yet we feel, too, that if the mystery that surrounds the study of embryology is ever to come within our comprehension, we must try not to be sentimental and have recourse to other means than description of the passing show. The recompense, we hope, will be to substitute a more intelligent interest in place of the older emotional response to the order of nature.

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CHAPTER I

INTRODUCTION: THE EXPERIMENTAL METHOD

The recognition of the advantage of applying the method of experiment to problems of development may be said to have begun with Wilhelm Roux in 1883. Today the need of this procedure seems so obvious that we are apt to forget the strong opposition that the movement at first met from embryologists of the old school, who had already developed a philosophy dealing with the developmental process as a series of historical events. The phylogenetic school had interpreted each stage in development as the survival of an ancestral adult form, in line with the then current theories of evolution. The study of development had resolved itself into a quest for ancestors for the successive stages. The fascination of that pursuit will probably never be felt by the oncoming generations. The inspiration, if it was an inspiration, led to a prodigious amount of observational and descriptive work. In a relatively short time the development of almost every kind of animal became known. Any modern textbook on "Embryology" will reveal the enormous amount of information that was gained by direct observation, often of the most detailed and refined kind. Little by little the myth that led to all this effort disappeared; but there had been formed a compendium of embryological facts that stands today on as sure a footing as any other body of information having to do with the visible structures of living things. It furnished objective materials and problems for the "experiments" described in the following pages.

The first attempts to construct a new philosophy of development on the basis of experimental work were not entirely free from still earlier speculations which had attempted both to

formulate a single all-embracing problem, and then, with quite insufficient data, to solve it. On the contrary, today we find ourselves face to face with problems at every stage of the developmental process. Have we lost sight of the main problem and become confused by the details? It may be worth while to look over the present situation and attempt to find out whether or not there is any one theme that is so much more important than the rest that it may be called the central problem of development, and if so, whether we are ready to make a frontal attack as in the past.

The development of most eggs begins with fertilization. The simplest interpretation is that the spermatozoön brings something into the egg that is necessary to start its development. It might seem possible to find out what the sperm contributes, but so far nothing of the sort has been discovered. On the contrary, some eggs can be started by altering slightly the composition of the sea water, but it does not seem probable that the sperm brings about a change comparable to this. Other eggs may be started by pricking them with a fine needle, or by subjecting them to momentary changes of temperature, or by merely shaking them. We are still in a quandary as to the meaning of this first phenomenon that we meet with in development, but at any rate the substitution of mechanical or chemical equivalents of the sperm reassures us that the problem is open to investigation, and removes the old idea of a mystical union of two essences during fertilization as the cause of development.

The fertilized egg begins to segment. It divides into two, then rests, and each cell or blastomere, so formed, again divides. How does the egg divide? Every step in the process in a transparent egg seems to be visible under the microscope, but we know that what we can see in this way in the living egg is only a fraction of what is going on in the interior. The internal processes were revealed by killing the egg, imbedding it in paraffin, cutting it into thin slices (sections) and staining the sections with differential dyes. Almost a new world of events became apparent. How far these interior changes are correlated with the division of the protoplasm remains still to be determined, but the imitation of the divisions of the egg by drops of oil, when local changes in surface tension are artificially brought about, leads us to think that the mechanics of division may some day be made plain.

When the egg has divided itself into a large number of cells and has given rise to a hollow sphere or blastula, a new series of events is inaugurated. The wall of one hemisphere turns in to make the digestive tract, and other internal organs of the future embryo. Once more we can appeal to models that will turn in if certain things are done to them. The analogy is striking; but the egg turns in to best advantage if it is let alone in its natural environment. It is the autonomous nature of the process that is its chief virtue. Here is another puzzle, probably also a mechanical one.

From gastrulation onwards change follows change. Extensive movements of groups of cells take place. Thickenings and outpushings appear here and there, to form eyes, arms, legs, gills. The spherical embryo takes on new shapes. The movements can be studied by staining the different regions with dyes that do not diffuse or injure the cells. The relation of the thickenings to neighboring regions can also be studied by removing certain regions and grafting them into other regions of the same, or of other embryos. By these means we are slowly getting information as to the relation of the parts to each other, and as to the meaning of location. Finally, sometimes early, sometimes late in development, the individual cells, or more often great groups of cells, begin to take on different shapes, and different functions. The embryo becomes differentiated. Again we are faced with a multiplicity of problems; for the differentiation that is taking place in one organ need not be the same as that which makes another. Each process must be unravelled in turn. Already methods have been devised that promise to throw light on some of these changes.

One of the most surprising results that experimental embryology has revealed has not yet been referred to. If an individual egg is cut into two fragments before fertilization, each fragment may be fertilized and may produce a whole embryo. One fragment will contain the egg-nucleus which harbors one set of chromosomes, the spermatozoön brings in another set (as in normal development). The two sets combine into a single nucleus that has the diploid number of chromosomes. Owing to the division of each chromosome by longitudinal splitting each daughter-cell at the first division also gets a full set. The process repeats itself, as in the normal egg, every time a cell divides.

The other fragment, the one without the egg-nucleus, if entered

by a spermatozoön, may also divide, but, since it receives only one set of chromosomes, all of its daughter-cells will contain only one set, unless by chance the number is doubled before the fragment itself divides, as sometimes happens. The half number suffices to produce an embryo that in all other respects is a duplicate of that arising from the nucleated fragment.

It is not, however, the presence of one or of two sets of chromosomes that is the unique feature of the situation, but rather the fact that a piece of an egg is capable of developing as does the whole egg. This property is not confined to the egg-stage, but if the first two cells are separated, each may develop into a whole. Some eggs can do this, but others cannot. In the latter case, the separated blastomeres form each a half-embryo. The property of restitution, or repair, is not confined to the egg or embryo, but may take place in some adult animals. It is then called regeneration.

This phenomenon has been singled out as furnishing crucial evidence that the egg, and even the organism, is not a machine; and the further inference made that the essence of the developmental process is not mechanistic or at least that the mechanism is always under a central guiding purposeful principle, which Driesch, who is the foremost advocate of this philosophy, has called the entelechy. If this is conceded, there is really a central problem of development, for, even though the means by which development takes place is conceded to be mechanistic, it is directed by something superior to mechanism residing in it, or brooding over it, that directs the machinery in such a way as to lead to a specific end-product.

This is not the place to discuss these questions at length, but it may be pointed out that, as long as the evidence or "proof" of vitalism rests its case on our inability to explain, at the present time, all of the phenomena of development, it seems worth while to continue our experiments in the attempt to push back the obstacle erected by the vitalist which may turn out to be purely visionary. So long as our work continues to yield new information, it seems preferable to occupy our time in dispelling a part of our gross, objective ignorance concerning living material, rather than to spend the time in discussions of a logical dilemma, especially when the alternative to a mechanistic interpretation offered by

the vitalist seems as difficult to understand as the phenomena that he pretends to "explain."

The words machine, or machine-like and mechanistic, do not necessarily have the same connotations, if by machine we mean the kinds of mechanisms that man has devised to carry out specific kinds of work. Furthermore, it is sometimes implied, that the adult organ functions as does a machine, but that the developmental process includes something more than this, because the embryonic machine makes a new machine (stage) and this again another one, etc.

There is something here, perhaps, that needs further clearing up. We ordinarily think of a machine as a mechanical device that receives something and turns out something else. The first something may be raw materials and the second steel rails. Here the materials that pass through do not enter in as a part of the functioning of the machine; they never become a part of the machine. We think of the machine as being the same at the end as at the beginning, except for wear and tear. The adult organism may seem to do exactly this kind of work when it functions, but this is too naïve a way of interpreting what goes on in the cells of the body which are often acting, not as physical but rather as chemical machines. In the latter case the raw materials that are being transformed may become at times an intimate part of the machine, in which case it does not remain the same as before, but develops into something else, at least for the time being. But when a particular function has ceased, we think of the organic machine as having returned once more, approximately, to its first condition (barring wear and tear, and excepting possibly the excitations in the central nervous system). There are also machines, both physical and chemical, that function in this way. In the telephone, for example, the energy that enters in becomes part of the machine, since the electrons of the wire are themselves carried along—or, as we say, carry the current. Chemical machines approach still more nearly the functioning of the organism. A gas flame, for instance, exists owing to the changes that take place in the incoming and outgoing materials. The materials and their transformation constitute the flame. A whirlpool or a thunderstorm are physical occurrences that also

offer analogies to the organism. These may even be divided and each constitute a new whole machine of smaller size.

We look upon a developing egg as an irreversible process, but whether a process be irreversible or not has really no important bearing on the question of mechanism at stake; for we know that chemical processes are sometimes reversible sometimes not. Therefore, when we say that the unique feature of the developmental process is that each machine (stage) develops a new machine (stage), we may be saying no more than that we are dealing with a series of irreversible reactions taking place in all parts of the embryo, except in those cells that are set aside as germ-cells which remain unaltered in this respect at least.

There is also abundant evidence showing that the early cells of the embryo are not always irreversibly determined, and that their power to become changed varies in different tissues. The comparison, then, between the process of development and the actions of a machine does not lead to a contradiction, if we interpret the word machine to mean something more than a mechanical device to make something, and look upon development as largely a series of irreversible chemical processes.

The cyclical nature of living things has often been referred to as a reversible process that is peculiar to them rather than to inorganic nature. The egg produces an adult which in turn produces eggs, etc. Weismann is generally credited with the solution of this paradox by his theory of the isolation of the germ-plasm. On this view only a part of the germinal material develops in each generation, or differentiates to become a new individual. The rest is set aside for future use. This capital set aside possesses the common property of living materials of increasing itself. Thus the cyclical nature of the phenomenon may seem to disappear; but the problem has only been changed into another problem, namely, as to how each time that an egg develops only part of it goes forward to become differentiated. As a matter of fact, neither the former nor the latter question, as stated, attracts much attention at the present time, because we suppose that the cells of the body, as well as those of the germ track, contain the sum-total of the inherited elements. The problem is not any longer why some of the first formed material is left behind to become germ-cells (or set aside to become germ-cells), but how, while retaining the total potentialities of the egg, regional dif-

ferentiation takes place to produce the different parts of the body. It is this latter problem that engages the serious attention of the student of embryology. He knows that in a given environment each kind of egg is set to run a specific course to its goal. The first problem would seem to be to find out what is given in the egg. The egg appears to be a relatively simple affair, but experience has taught the deceptiveness of this apparent simplicity. Experience has also taught the embryologist to be on his guard against assigning to the egg imaginary complexities expressed in such phrases as polarity, symmetry, organ-forming-germ-regions, morphogenetic processes and differential cleavages. These terms, some of which may be useful in a purely descriptive sense, become deceptive, when, by a sort of inversion, they are employed as though something was explained by them. The whole literature of embryology teems with phrases of this sort, which, while they may not deceive the writer, may often leave the reader under the impression that an attempt has been made to explain something. In reality, some obscure event has only been given a name that conveys no more meaning than the event itself.

As has just been said, the assumption that the egg is a very simple affair may be as misleading as the contrary assumption that it is very complex. Neither the one nor the other statement means much until it can be stated in what sense the egg is simple or complex. It is easy to juggle with words in this connection, for in one sense the visible structure of the egg is obviously simple in comparison with the structures of many kinds of cells of the individual that develop from the egg, but genetic work has shown that each chromosome of the egg contains a very large number of minute bodies, the genes, presumably each different from all the others, arranged in linear order. Each gene is involved in the development of the characters of some one or more parts of the body. Moreover, it is probable that the materials produced by the genes interact, first or last, on the development of many, or most, or all of the parts of the developing embryo. Numerous though the genes may be, thousands perhaps in number, yet the end-product due to their interaction may be vastly more complex than the sum of all the genes.

There is another consideration derived from genetics that has

an important bearing on the problem of the relation of the genes to embryonic development. The arrangement of the genes in the chromosomes bears no relation at all to the arrangement of the parts which the structures of the fully formed individual bear to each other. The developmental changes that we see and try to explain are supposed not to be primarily due to changes in the chromosomes (for the genes remain, we think, intact and perhaps unaltered throughout embryonic development), but in the cytoplasm of the egg. These changes in the cytoplasm are relatively gross processes in comparison with the minuteness of the genes that are the ultimate agents behind them. In the study of embryonic development we see only the gross events; the presence of other agents is inferred from a different kind of evidence. How, then, is the ordered sequence of events, that takes place in the cytoplasm, related to the activity of genes in the chromosomes if there is no correspondence of arrangement in the two? Is it due, for example, to the sequence in which the genes become active? We dare not make any such assumption, tempting as it might be to do so, for there is no evidence that there is any such sequence in activities of the genes. To make this assumption outright, while seeming to account for the result, would beg the whole question and leave us still as much in the dark as before. If we could get evidence of a correlation between each new advance in the cytoplasm and a subsequent effect on the genes themselves, we might then make such an assumption. For, if it be admitted that the reaction of the genes is affected by the changes in the cytoplasm, then a sort of harmony might be imagined to exist between them. But even if this were true, it is questionable whether the hypothesis would be much better than one that calls for no such correlation, but assumes that the genes are active all the time, and that the effects they produce are different at each stage because each stage is itself different from what went before. We have no evidence that helps us to decide between these alternatives, and for the present can better direct our studies to problems more open to examination.

For our present purposes we need not go behind the evidence showing that the genes may change the cytoplasm and the cytoplasm then acts in a specific way. Our immediate problem is first to find out the nature of the changes that take place in the cytoplasm. Having done this, we may then possibly be in a better

position to find out the way or ways in which the genes have altered or affected the cytoplasm so that it behaves in certain obvious ways. At the same time it may happen that the evidence concerning the way the genes affect the cytoplasm will come from the genetic work itself rather than from embryological experiments. The problem can well be studied to advantage from both ends.

In the cytoplasm of the egg there must reside at the beginning of development those factors that start it on its prescribed course. It is possible, but whether probable or not we cannot say, that at this time only those properties are present that determine the kind of cleavages that take place. The later stages would then depend on further changes resulting in part from those that have preceded them in the cytoplasm, in part from the continued action of the genes. We have evidence, however, that even when the sperm brings in chromosomal genes that may be supposed to affect the first stages of the development of the egg, there is no immediate effect on the egg that has been formed under the influence of a contrasting set of genes. But other genes brought in by the sperm, that relate to later embryonic characters, may affect the characters when they appear. It takes time, then, for the genes to act and if this is true, this condition may turn out to be very significant, for it concerns the nature of the action by which the genes affect the cytoplasm.

If, then, as has been said, the arrangement of the hereditary elements in the chromosome bears no causal relation to the arrangement of the parts of the embryo, is it necessary to assume that the original arrangement of the cytoplasmic materials of the egg have, at the beginning, a predetermined relation to the prospective embryo? Even this is apparently not the case, or at least only in a very general way. For, here, we have explicit evidence showing that when the egg is cut into fragments, each fragment may make a whole embryo. Furthermore, there is excellent evidence that in some eggs the median plane of the embryo is not predetermined in the egg, but that any meridian may become the median plane. This means, of course, that within specified limits almost any part of the egg may become any part of the body. If this evidence be accepted as sufficient we are dealing with a rather peculiar situation, yet the evidence just stated must not be taken for more than it is worth; for, granted

that almost any part of the egg if sufficiently large may form a whole embryo, it does not follow that the fragment is homogeneous throughout, or, even if it is nearly so, that it may not be given a set or arrangement by some extraneous event such as the entrance of a spermatozoön at one point or by the local development of an aster at any one region in the interior. Either of these events might, then, set in train a series of processes implicit in the composition of the material of each kind of egg, whose primary directions may have been initiated by something outside.

The evidence at hand, while not entirely adequate, permits us, I think, to picture provisionally the situation in some such way as sketched above. It by no means suffices to tell us what we need to know about the kind of process we are dealing with, but it is enough perhaps to suggest that we may accept the evidence at its present value and proceed to discover if possible the kinds of changes that take place in the egg, or even in a fragment of the egg, without making any assumptions other than those consistent with physical and chemical phenomena.

The essentials of the preceding argument may be summed up as follows:

The localization of the embryo-forming regions is not given, either in the order of the genes or in the regions of the unfertilized egg already laid down. The localizations take place progressively. The orienting points or planes may in part have an extraneous origin, such as the point of entrance of the sperm, or be impressed on the egg by the surrounding coat, as in the insect egg; or, as in artificial parthenogenesis, by the accidental development of a division center in some one region of the egg. Once set in motion the subsequent events may to some extent and within exact limits be subject to readjustments, but ordinarily one event, carried through, becomes the starting point for the next event. The determination of the sequence may be strictly within very narrow limits. All this is determined by the composition of the material of each kind of egg, not so much by the distribution of the parts of the egg before fertilization, as by the sequence of events that follow fertilization or its equivalent. Whether it is necessary to assume some simple element of direction or physical arrangement as an essential property of the unfertilized egg or of any of its parts is a mooted question at the present time. Even such a simple

assumption need not mean that an element of direction is present before development begins, but rather that it progressively appears as events take place. For example: the cleavage pattern of a fragment, which is identical with that of the whole egg, cannot be imagined to be present at first in the fragment, but must appear as the cleavage progresses and be chiefly dependent on each successive stage. If, then, we cannot imagine the pattern, as such, inherent in the fragment, we need not assume it to be present in the egg. On the other hand, the egg has usually passed through its earlier phases in connection with other cells or parts of the maternal body, such as the walls of the ovary, and has a visibly stratified arrangement of its parts, i.e., it is not homogeneous. Moreover, in many cases this arrangement, often spoken of as polarity, is carried over to the ripe egg, and the normal changes and subsequent events are intimately correlated with this structure. The visible stratification might suffice to give a simple point of departure for one, at least, of the directions of differentiation, but we know a little too much to build on such an assumption; for, pieces from any part of the egg may also develop normally. To meet this situation it has been commonly assumed that the visible "polarity" has behind it an invisible arrangement of some imagined basic material of the egg. Whether this assumption suffices to cover the case will depend, in part, on whether the evidence shows that the fragment does retain the original visible directions and builds on this as a foundation, or whether it acquires new directions along the lines of the old ones, or quite independently of them. This is a question that can best be discussed in connection with specific evidence indicating that the polarity, if it exists, may be affected by extraneous agents.

The question was raised as to whether there is one overshadowing problem of development. Granting that there may be a multiplicity of mechanical problems that remain to be solved, we may still be asked the question whether any one of them appears more unique, or characteristic, or insistent than any of the others. Speaking for myself, I doubt if any such selection can be profitably made at present. When we have found answers to some of the problems that have been mentioned—and there are a great many more—we may decide that some are more important than are others, but, at present, it seems to me that modesty alone would

disparage any such attempt. It is true that, since the study of embryology has been largely in the hands of morphologists, the problem of the changes in form through which the embryo passes will seem to them to be the central theme of embryology. On the other hand, physiologists who have studied development have not been enamored of this problem, but have ignored it, either because, I suppose, they were not interested in it, or did not realize its significance, or else because they supposed that the study of living matter has not reached a point where such questions can be profitably discussed. Even if the diagnosis of the morphologist should prove to be nearer the mark, enough work has been done to show that before we can hope to study experimentally the changes in form of "the embryo as a whole," we must first undertake to work out the many kinds of changes that are involved in each separate change in form in order to synthesize these under one or more general headings. It seems, then, more worth while to drop the old fruitless discussions of the meaning of development, and turn to the many problems that have already presented themselves at almost every stage of the process.

The application of the experimental method to problems in development is itself an interesting problem. In physics and chemistry the experimental method has reached such a degree of perfection that it is possible to speak of crucial experiments—experiments designed to give an answer in terms of better understood relations. In embryology, on the contrary, the experimental work appears crude in comparison. Nevertheless, the more exact experimental sciences have also passed through such beginnings, and on the borderland of physics and chemistry, where new principles may be met with, the experiments may also be little more than a preliminary testing out of possibilities. In fact, it might be argued, I think, that the famous *experimentum crucis* is something of a bogie, for it is often not so much a method of research as a final and triumphant demonstration when all the pioneer work has come to a successful ending.

It is not, I think, a disparagement of the experimental method in embryology to admit that, at its beginning, it is still little more than the adventurous exploration of a new field. The exploratory feature of preliminary experimental work often serves to suggest problems rather than to interpret them. In fact, it is often little more than an extension of observational work. Many of the

experiments to be described in the following pages find their chief significance in suggesting new problems in development. While even such a simple experiment as that of separating the first two blastomeres has not answered any fundamental problems, it has revealed to us unsuspected possibilities in development.

In recent years a good deal has been said about the importance of quantitative methods in biological research. The impression is sometimes given that the value of a piece of work is commensurate with the number of measurements that it contains. No one will deny the need of exact measurement in terms of some objective unit, but the value of a contribution of this kind may bear no relation either to the number or the exactness of the measurements that it contains. In themselves, the measurements may be as empty of significance as any other kind of descriptive materials. The statistical answers that can be wrung out of such measurements may have very little meaning if the quantities measured depend on a multiplicity of causes. Statistical treatment may indicate that certain results are significant, but what they signify no man may know, or even surmise. But when the materials measured have been made as uniform as possible, which means that the condition under which the differences measured have been largely controlled, then the results will give a significant answer. Experience in the physical sciences has shown that reliable data are those obtained by exact measurements. The nature of the material and the use to which these measurements are put is the important feature of the scientific procedure.

The working hypothesis is another indispensable tool in experimental science. Essentially it is an attempt to find an answer to some feature of a complex situation in terms of better understood relations, which, from a mechanical standpoint, means reference to accepted chemical or physical principles. It will be observed without further elaboration that hypotheses, as here interpreted, can have little meaning without reference to the more advanced physical sciences; for, without reference to a better understood background they become mere speculations, however logical their presentation may appear. The justification of their use in the physical sciences is found in their fruitfulness and suggestiveness in the progress of research. This is conceded in physics and chemistry and enough has been done even in the biological sciences to warrant their employment.

It is repeatedly said that there is no experiment without a control. This slogan, too, may be misunderstood. In any simple physical experiment, where all the conditions are known, experiments are carried out without any control, because the conditions of the experiment are themselves controlled. The need of a control arises in cases where many or all of the conditions are unknown. Then two situations are brought about, that differ from each other in one known condition (that may be simple or complex). The different results obtained are attributed to the differences in the two situations, and one or the other condition is said to be the cause of the difference. In preliminary experimental work this is often a valuable and indispensable means of finding out more about the environmental or internal conditions under which certain events do or do not happen. It is often an essential part of discovering the factors present in a complex relation, but when the conditions have become simplified, the control, as such, may be no longer needed. This occurs in the famous *experimentum crucis*. In experimental embryology we are far from having reached this happy consummation, and as the following pages will only too well reveal many of the "experiments" do little more than describe what happens to the developing egg or embryo under a variety of new conditions imposed on them by the investigator, in the hope they will do something that may throw light on some of their properties not revealed under the conditions in which they "normally" develop.

CHAPTER II

FERTILIZATION AND CHEMOTAXIS

It is customary to think of the germ-cells, the egg and the spermatozoön, as essentially undifferentiated cells, which, after union, furnish the starting point for a new body of differentiated cells. In fact, however, both the egg-cell and the sperm-cell have already become differentiated when ripe. They are in some respects highly specialized cells both in structure and in function. They carry, it is true, the sum total of all the inherited elements of the species, but they carry only one set of elements (genes) while all the other cells including the early germ-cells themselves carry a double set.

The germ-cells (gametes) like the body-cells have become specialized for particular functions. The egg-cell stores up foodstuffs, and when ripe receives a spermatozoön. It then proceeds to divide its material into a large number of small cells out of which the embryo is formed. The sperm-cell is a locomotor cell. It punctures the surface of the egg, and its head, which is largely the nucleus of the original cell that developed into the spermatozoön, enters and approaches the egg-nucleus. The union of the haploid egg-nucleus and the haploid nucleus of the spermatozoön restores the full (diploid) number of chromosomes to the egg.

In some species of animals the sperm is supposed to bring in a formed body, the centrosome, which after division becomes the division-center of the egg. In other species it is supposed that the first centrosome is formed in the cytoplasm of the egg under the influence of the sperm-nucleus, and in still other cases it has been said that the sperm supplies one centrosome, the egg another, the two becoming the poles of the karyokinetic spindle of the dividing egg.

The eggs of many aquatic animals are fertilized as soon as they have been set free in the water. This somewhat casual pro-

cedure is supposed to be regulated to some extent by substances simultaneously set free by the female (or possibly by substances excreted by the eggs themselves), which acting as a stimulus on the male call forth an immediate ejaculation of his semen. In other cases it is supposed that the semen of the male when set free excites the female to ovulation. Such relations, whenever they exist (there is little really critical evidence to support them), would be a step towards conservation, and in advance of the more wasteful procedure of setting free the eggs and sperm at random.

In other animals, more especially those living on land, the semen of the male is introduced into the oviduct of the female by way of the genital opening, which serves at the same time as an outlet for the eggs after fertilization. The spermatozoa, becoming active in the oviducts, fertilize the eggs as they enter or pass through these tubes. This occurs in some of the worms (nematodes) and in the higher vertebrates. In other cases the spermatozoa may be stored in special receptacles as in the earthworm and its relatives, in gasteropods (both land and water forms), and in most insects. In the insects, only a few spermatozoa at a time are set free as the egg passes by the opening of the sperm receptacles. There may also be glands connected with the oviducts whose secretion, poured out into the oviduct as the egg passes, serves to dilute the mass of sperm or to excite the sperm to activity. In the higher vertebrates the sperm from the vas deferens is mixed with secretions of the prostate and other glands of the male that excite the sperm to activity.

There are several other methods of insemination, such as the hyperdermic injection of the sperm into the tissue of the female in some of the flatworms, rotifers, polyzoa and leeches; the deposition of packets of sperm on the substratum as in some salamanders or in depressions of the covering of the female as in the crayfish, or in bundles inside the mantle of the female as in the squid and octopus.

In spiders a drop of sperm is transferred by the palp of the male to the genital orifice of the female. In the octopus an entire arm is thrown off (autotomized) carrying bundles of sperm, after it has been thrust into the mantle chamber of the female where later the spermatozoa leak out and fertilize the eggs as they issue from the oviduct.

In nearly all cases of external fertilization the spermatozoa are elongated or thread-like in shape, moving by means of a vibratile tail. When fertilization is internal the spermatozoa often have a similar shape and move in the same way in the fluid over the inner wall of the oviduct. An exception to this rule is found in the amoeboid sperm of nematodes.

When a coat of jelly surrounds the egg the spermatozoa bore their way through it, apparently by the same kind of activity by which they swim. In some animals the spermatozoa can enter the egg at any point of the surface, while in others the spermatozoa can enter only in the polar hemisphere (frog), and in still others they enter only at the antipolar hemisphere (some molluscs and ascidians).

In eggs with a tough membrane (some nematode's, insect's, squid's and fish's eggs) the spermatozoa can enter only at one point of the surface where an opening in the membrane—the micropylar opening—makes possible their passage. In the decapod crustacea there is a cork-screw-like arrangement on the spermatozoön that enables it to pierce the egg-membrane (that is, without a micropyle) and, through the opening thus made, the sperm nucleus is projected (or is drawn) into the protoplasm. The final step, the absorption of the spermatozoön into the protoplasm of the egg, appears to be brought about to a large extent by the action of the protoplasm itself. In fact, the sperm's own activity may cease as soon as its tip has penetrated the surface of the egg.

In many animals the eggs are not fertilized until they have been discharged from the body. In a few cases the large germinal vesicles may be present when the egg receives the sperm, but in most cases the egg does not normally receive the sperm until the egg-nucleus has disappeared, and the spindle of the first polar body has been formed. In still other cases, as in the sea-urchin's egg, both polar bodies have already been given off when the egg is set free to be fertilized.

For each species there is a special stage at which the maturation of the egg comes to a standstill, remaining in this condition until a spermatozoön enters. In some species this stage is reached while the eggs are still in the ovaries or in the oviducts, in other species after the egg has left the body. The changes in question involve the nucleus and chromosomes rather than the cytoplasm, but there can be no doubt but that cytoplasmic movements are in-

volved in the breaking-down of the nuclear wall and in the migration of the chromosomes of the first maturation spindle to the pole of the egg. This variability in the ripening process is a clear indication that the egg when fully formed is ready to begin its development, and in fact it may be induced to do so if the inhibition or block, that brings these inaugural changes to a standstill, is removed. The entrance of the spermatozoön serves to remove the block and allow the development to proceed. In natural parthenogenesis there is no block, and the maturation process is continuous with the developmental phase. In some species there is a "facultative parthenogenesis" which means that if an egg, that is generally fertilized before development, is not fertilized it may begin to develop. Artificial parthenogenesis may be induced by removing the block that normally holds the egg in check.

The eggs of some animals must be fertilized very soon after they have been discharged from the female, if normal development is to follow. It was found by Reighard ('93) that the best results with the eggs of the wall-eyed pike are obtained if they are fertilized as soon as they are set free in water. After two minutes 40 per cent of the eggs segment; after four minutes only 17 per cent; after six minutes 10 per cent, after eight minutes 5 per cent; after ten minutes no eggs segment. For many fish "dry fertilization" gives the best results. Dry fertilization consists in stripping the female, to force out her eggs, and then in squeezing the milt (semen) of the male directly over them. A few minutes later the eggs are placed in water. The eggs of some marine fish (Ctenolabrus) are entered by only a single sperm if the semen is added at once to the eggs when they are set free in sea water; but if fertilization is delayed for only a few minutes several sperms may enter and cause abnormal development.

In a marine annelid, Platynereis megalops, in which fertilization is normally internal, Just ('15) found that artificial fertilization outside the body may be brought about if sperm and eggs are mixed dry, i.e., without contact with sea water. If the eggs are first placed in sea water, for even a few seconds, fertilization does not take place. The sperm may, however, first be set free in sea water, and then if the water is filtered off, the residue (sperm) will still fertilize the eggs. Whether the result here is due to some injurious action of the sea water on the eggs that prevents their fertilization, or whether the result is due, as Lillie and Just

both think, to the removal by the sea water of some substance (fertilizin) that must be present in the egg if it is to be fertilized, has not been certainly demonstrated.

Does the Egg Attract the Spermatozoa?

Practically all the experimental work on fertilization has been done with spermatozoa that swim freely in water before they reach the egg. In fact, nearly all this work relates to a few species of sea-urchins and starfish. This is owing to the abundance of these echinoderms, and the ease with which the eggs and sperm can be obtained in great quantities.

When the sperm of the sea-urchin is added to sea water containing the eggs, a cloud of sperm quickly accumulates about each egg, making a halo around the jelly. The picture thus presented led naturally to the conclusion that the egg attracts the spermatozoa. This view was held by nearly all of the early observers and by some later ones also. But more critical work has not established this interpretation.

The supposed attraction of the egg for the spermatozoa has often been said to be due to some sort of chemotaxis. This term means that some chemical substance is set free by the egg that causes the sperm to turn toward the source of diffusion. The experimental evidence for such a reaction is very meager, for the method of swimming of the spermatozoa makes it difficult to explain how chemotaxis could be called into play. There are only a few observations that give an exact account of the way in which the sperm swims. The more accurate are those on the sea-urchin's spermatozoa (Buller, Lillie, Winslow). These spermatozoa swim in a spiral path, turning on their own axes as they move forward, making one revolution in each spiral. To judge by analogy with spirally swimming protozoa, the rotation is due to an asymmetrical form of the sperm. The lashing movement of its tail is supposed to drive it forward; its asymmetrical form to make it rotate in a spiral path. The problem is whether a chemical substance, diffusing from a center of higher concentration, could act as a directive stimulus on a passing spermatozoön in such a way that the spermatozoön turns towards more and more concentrated regions of diffusion; for, the spermatozoön is moving in a spiral,

and every instant turns a different side towards the more concentrated region.

Many protozoa also swim in spiral paths. According to Jennings' ('06) observations when a drop of 1/10 per cent sodium chloride is introduced into a ½ per cent solution of sodium chloride containing paramecia, the animals soon collect within the drop. Such an accumulation resembles to some extent the accumulation of spermatozoa around the egg, but Jennings has shown that the result in paramecium has nothing to do with chemotaxis. If an individual paramecium is closely watched one will see that if it enters the drop at random it swims straight into the drop. No reaction takes place. But when it reaches the far side of the drop, and is about to pass out of it, a characteristic motor response takes place. The paramecium stops, backs into the drop, (rotating as it moves) and swerves towards its aboral side. It then moves forward in the drop in a new path. Again it reaches the edge and again the motor reaction takes place. It is caught in the drop which acts as a trap. In a short time, all the paramecia that have entered the drop, by chance, are caught. Evidently the accumulation is not due to chemotaxis.

On the other hand it has been shown that certain spirally swimming protozoa do orient themselves toward or away from a source of illumination. Here according to Mast's ('11) observations the response takes place at a definite point of the spiral, when, for example, one side is turned toward (or away from) the source of stimulation (light in this case). The response is so timed in the swerving of a positive organism that the movement is toward the light. The result is, therefore, not haphazard, but directive. It leads quickly to an orientation toward the light. When so turned no further reaction takes place, because, at each rotation the anterior end (where the receptive organ is situated) is equally illuminated throughout each turn of the spiral. If the spermatozoön were so constructed that it could also give a motor response to a chemical gradient at a definite point of each rotation, it is conceivable that it might turn from a region of lower to one of higher chemical concentration; but spermatozoa do not give a motor response of this kind. If it occurred it would probably have been seen. There is, moreover, no structure known in the sperm that suggests the presence of an organ responsive to chemical gradients. Again, the difference between the concentra-

tions on the two sides of the minute sperm would be so small that it does not seem possible that it could react to this difference. More convincing is the fact that careful observations fail to show that the spermatozoa turn toward the region of higher concentration as they pass across a diffusion field. On the contrary, there are several observations (Buller) that seem to show that the spermatozoa do not turn, but pass straight across such a field and out again on the other side.

None of these arguments may seem conclusive, but taken together they make it appear improbable that there is a chemotactic reaction to a chemical gradient by spermatozoa. There are, moreover, other observations that suffice to furnish a reasonable explanation of the gathering of the sperm about the egg.

Sea-urchin eggs are surrounded by a jelly. The spermatozoa on coming in contact with the jelly stick to it. Their further activity carries them into it. In a relatively short time all the free-swimming sperm are caught in the jelly. This would seem to suffice to explain the cloud of sperm seen at the surface of the jelly without any further assumption. Any sperm, whose path through the jelly is such that it comes into contact with the surface of the egg, calls forth a reaction at the surface that leads to the engulfment of the head of the sperm. The first one to reach the surface usually fertilizes the egg. Others are prevented from entering, either because an impenetrable fertilization membrane is formed around the egg after one sperm has penetrated, or because the egg no longer responds after the reaction to the first sperm has taken place.

There is an old observation of Pfeffer's ('84) relating to the fertilization of the oösphere of ferns by the free-swimming spermatozoids, that has often been quoted as demonstrating a chemotactic reaction. The oösphere lies at the bottom of a sort of chimney whose interior is filled with a semifluid (gelatinous) substance containing malic acid. The spermatozoid is a coiled filament with long cilia that cause the body to rotate as it moves somewhat erratically forward. Pfeffer found that the spermatozoids soon collect about the opening of the chimney and on entering it reach the oösphere at its bottom. He inferred that the spermatozoids are attracted by the malic acid. Further experiment with capillary tubes filled with a weak solution of malic acid supported this view. When such tubes were placed in a drop

of water containing spermatozoids they accumulated around and entered the open end of the tube from which the malic acid was diffusing.

The question may arise as to whether the spermatozoids are attracted by the malic acid or whether those that enter the more concentrated regions are caught like paramecia in a trap. In favor of the view that they are attracted is the observation of the rapidity with which they collect at the mouths of the tubes containing a weak solution of the acid. Pfeffer's ('84) statements strongly indicate that the accumulation occurs so quickly that it seems improbable that the result could depend on the chance entrance of the sperms into the acid. Shibata ('05, '11) states, for Isoetes, that the spermatozoids collect quickly around the mouths of the tubes (50 to 80 mm. in diameter) containing sodium malate (1/100 to 1/1000 mol solution), and that, furthermore, the scattered sperms react suddenly to the diffusing acid, turning on their axes and steering at once towards the capillary tubes. In a few minutes many hundred sperms collect around the tubes. This lasts at most for 3 to 5 minutes and after 10 minutes none of the reacting sperms are found near the tubes. Inside the tubes they come to rest. Other observers have also stated that the sperms are attracted towards the tubes (Buller '00, Lidforss '05, Bruchmann '09, etc.). These observations seem to show that the spermatozoids of ferns and related forms orient to a center of diffusion, and although the observations still lack the precision as to the mode of orienting that one might desire in a matter of such delicacy, and although there are indications that the trap action may also play a rôle in the outcome, still, I think, the facts, taken as a whole, are in favor of a chemotactic response. Nevertheless evidence from this source can not be used in support of chemotaxis of animal sperm whose structure and mode of locomotion are quite different from those of the spermatozoids of ferns.

Strasburger ('87) stated that the egg of the marine alga, Fucus, attracts passing sperm at a distance of one and a half diameters of the egg. Bordet ('94) failed to find any evidence of chemotactic attraction of the egg of Fucus for the spermatozoa. He observed that the sperms stick to the surface of the egg by the tip of one of their two cilia, which accounts for their accumulation around the eggs. Buller ('02) states that his own obser-

vations did not reveal any certain attraction of the sperm from a distance. The collection on the egg was in consequence of their ability to cling to surfaces. Robbins ('16) tested Fucus sperm by means of Pfeffer's capillary tubes containing various substances. With a weak solution of HCl (0.1 mol) there was an accumulation around the tube and with a still weaker solution (0.01 mol) there was a collection within the tube. No change in the direction of the movements of the sperm swimming near the tubes could be observed. The results are explained as due to the toxic action of the acid on the sperm that came in contact with it. They were injured and ceased to move when they reached a lethal region.

An experiment by Loew ('03) is interpreted by him as showing a chemotactic response of the sperm in animals. He placed on one side of a slide a small piece of the lining of the uterus and on the opposite side a piece of muscle or liver. A drop of salt solution containing sperm was put between the two pieces of tissue and a cover-slip added, which, spreading the drop, causes it to come into contact with the two pieces of tissue. It was found that the sperm collected near the lining of the uterus. This tissue gave an alkaline reaction. He compared this result with a similar experiment in which the alkaline lining of the digestive tract was used. This also was found to "attract" the sperm. Even filter paper saturated with alkalies acted "chemotactically" as compared with the same paper not alkaline. It is not certain, however, that the result is due to chemotaxis. The sperm may be caught by the slime from the uterus or the digestive tract and hence accumulate on that side of the slide. It is not evident why the alkaline piece of paper should collect the sperm unless the solution was strong enough to kill or make quiescent those sperm that entered the solution.

There are some significant observations on the fertilization of the sea-urchin's egg (Echinus) by Buller ('02). The egg has a diameter of 0.11 mm., and is surrounded by a gelatinous coat 0.036 mm. when mature. The jelly swells in sea water until after 24 hours it is twice as wide as at first. The spermatozoön measures 0.051 mm. in length. It swims in a spiral course. The spirals may be so steep that the spermatozoön appears to swim in almost a straight line, or the incline of the spiral may be so gentle that the spermatozoa appear to be swimming in circles.

Those sperm that penetrate the jelly in a radial direction quickly reach the surface of the egg; others may take an oblique course through the jelly, but Buller's observations show that the direct (radial) path is the most frequent one. Hence it might appear that there is some influence emanating from the egg, that acts on the sperm which has entered the jelly in such a way that it turns toward the egg; but the following observation led Buller to conclude that no such attraction (chemotaxis) exists. Ripe eggs were killed in osmic acid and washed in sea water for half an hour to remove the acid. They were then placed on a slide in a drop of water to which sperm was added. The radial penetration was as evident as when the jelly surrounds a living egg.

Experiments of von Dungern ('02) brought to light some of the conditions affecting the fertilization of an egg by sperm of its own species and also some of the conditions that prevent the fertilization of eggs by sperm of other species. He found no evidence that sperm are attracted, even through the smallest distance, by substances set free from the egg. Von Dungern takes into account the activity shown by a spermatozoön in contact with a solid or a semi-solid body. They cease to move forward but stick to a surface, and swimming over it in circles fail to penetrate. This also happens when starfish sperm come into contact with the jelly of the sea-urchin's egg. But when the sea-urchin's spermatozoa meet the sea-urchin's egg a different reaction is observed. They do not circle on the surface of its jelly but take a radial position and then penetrate the jelly. That a substance is present that brings about this vertical position of the sperm was shown by von Dungern as follows: Eggs of Echinus (or Sphaerechinus) were ground up and mixed with gelatine. Small pieces of such gelatine were then placed in sea water containing sperm. Most of the sperm took up a vertical position to the surface of the gelatine on coming in contact with it, while in the control experiment—gelatine without egg substance —most of the sperm showed only a tangential or circular motion on the surface. The vertical position of the sperm is due, von Dungern suggests, to a substance that inhibits the ordinary contact reaction between the sperm and a solid surface. The substance is not, however, peculiar to the egg, but is present even in the spermatozoa; for, if the gelatine be made with them instead of with egg substance the living sperm will take a vertical position

on entering this gelatine. Von Dungern thinks that the substance in question lowers the contact reaction of the spermatozoa and leaves them free to enter the jelly of the egg in a vertical position *if they have approached in that direction,* which in most cases is the position of approach. He does not think the substance actually causes a spermatozoön that approaches obliquely to take a vertical position to the jelly.

Similar behavior of the sperm of certain insects in contact with the surface of the outer coat of the egg had been observed by Dewitz ('85) who supposes that such activity causes them to enter the micropyle of the egg. It may be, however, that those spermatozoa that enter at once into the micropyle are the ones that fertilize the egg and not those that circle over the surface of the egg coat, and that the contact reaction shown by them has nothing to do with the process of fertilization of the egg.

Massart ('88, '89) who observed the fertilization of the frog's egg concluded that the spermatozoa come in contact with the jelly by accident and then cling to it. He states that they bore radially through the jelly, their orientation being a response to the increasing density of the layers nearer the egg.

Fol ('75), who observed the radial penetration of the spermatozoa of the starfish, concluded that the direction of the spermatozoön is determined by fine canals that penetrate the jelly radially. The presence of such canals is doubtful, and, even if present, their diameter is less than that of the sperm head and could not account for the phenomenon. In other eggs, where no radial structure can be observed, the sperm are still able to penetrate the jelly and fertilize the egg.

According to recent observations of Chambers ('23), the passage of the spermatozoa of the starfish through the jelly of the egg is associated with extraordinary activity of the egg. He states that thirty seconds after insemination there may be seen amongst the many spermatozoa that adhere to the outer surface of the jelly, one or more whose heads are advancing into the jelly toward the egg. The tail trails motionless behind, or at most, occasionally lashes feebly to and fro. At the point of the surface of the egg towards which the sperm is travelling a conical elevation is present and from its summit a tenuous thread extends to the head of the advancing sperm. This thread of protoplasm has been projected from the egg toward that point of the surface

where a spermatozoön has touched the jelly. This reaction may happen in response to several spermatozoa at the same time, but not to all of them that stick to the surface. The most plausible suggestion he thinks is that a substance diffuses from the head of the sperm which, penetrating to the surface of the egg, calls forth a fertilization cone that sends out a filament. Chambers is inclined to think that the sperm is drawn into the egg by the contraction of the filament, because as the sperm advances the filament shortens. The head of the spermatozoön on reaching the fertilization cone enters it and passes into the egg as the cone subsides. The fertilization membrane appears around the cone before the sperm reaches the cone but an opening is probably left, pierced by the filament, and through this pore the sperm-head passes. These observations of Chambers are not in accord with what occurs in other eggs surrounded by a jelly, where the reaction of the egg does not take place until a spermatozoön has touched the surface. Lillie and Just ('24) have pointed out that the spinning activity is due to an abnormal condition of the egg, and that the changes observed by Chambers have no relation to normal fertilization.

CHAPTER III

ACTIVITY OF SPERMATOZOA

In most eggs that are normally deposited in sea water, the eggs may be fertilized even after several hours, or days. Their life may be prolonged, as Loeb has shown, by the addition of small amounts of potassium cyanide to the sea water. Potassium cyanide also inhibits oxidative processes in the egg, and it seems not improbable, therefore, as Loeb suggests, that oxidation is one, at least, of the changes that lead to the death of the egg unless it is fertilized. This conclusion may seem paradoxical when it is recalled that immediately after normal fertilization the oxidation processes in the egg are suddenly increased, but this may mean that the oxidative changes that take place in the unfertilized egg involve a different set of changes from those that take place after fertilization. The suggestion has been made by Gorham and Tower that the effect of the potassium cyanide in prolonging the life of eggs is only to prevent the development of the bacteria or other organisms in the sea water that produce substances injurious to the egg, but while this may be a contributory factor, it is probably not the essential one that prevents the unfertilized egg from deteriorating.

The time-limit of the fertilizing power of the sperm differs greatly in different animals. In a general way it may be said that spermatozoa can function only so long as they are active. Unripe or inactive sperm will not fertilize unless aroused to activity by external agents. How long spermatozoa remain active depends partly on themselves, partly on external conditions. As early as 1785 Spallanzani made some observations on the effect of temperature on the activity of spermatozoa. Prevost and Dumas in 1824, and Newport in 1854, were also interested in their activity. Koelliker, in 1856, carried out an elaborate series of experiments on the effect of external agents that arouse sperm to activity or bring them to rest. Many scattered observations

in this same field were made between these early dates and more recent times when more accurate measurements began to be made and chemical relations to be discussed.

Gemmil had observed in 1900 that the length of the functional life of spermatozoa is dependent upon the dilution of the suspension, and he concluded that this was due to their exhaustion resulting from their own activity which is in proportion to the amount of sea water that excites them to activity. In other words, their store of energy is exhausted in proportion to their activity. Later Cohn ('18) made accurate determinations of the relation between the amount of dilution of the sperm of the sea-urchin and its power to fertilize the eggs. The next table (Table I) gives the data. It will be observed that in 4 per cent concen-

TABLE I

THE LENGTH OF LIFE, AS MEASURED BY THE FERTILIZING POWER OF SPERM SUSPENSIONS OF DIFFERENT CONCENTRATION

Date	Time of Insemination	Age of Sperm		1	2	3	4
				Concentration of Sperm Suspensions			
				4%	1%	0.5%	0.25%
		Hours	Minutes	Percentage of Eggs Fertilized When 1 Drop of Sperm Added to 5 Drops of Eggs in 10 cc. of Sea Water at Intervals as Noted.			
7/25	12:20 A.M.	0	0				
7/25	2:30 P.M.	14	10	100	98	67	10
7/26	12:00 M.	23	40	100	98	15	0
7/27	11:20 A.M.	47	00	100	0	0	0
7/28	12:15 P.M.	71	55	98	0		
7/29	8:20 A.M.	92	00	85			

tration the spermatozoa fertilized most of the eggs after nearly four days, while in ¼ per cent concentration they failed to fertilize any eggs after 14 hours.

Cohn measured the total carbon dioxide production in sperm-suspensions by determining the hydrogen potential of the suspensions as a function of time (Table II, and Diagram I). The length of life of the different suspensions as computed from

TABLE II

HYDROGEN POTENTIALS OF SPERM SUSPENSIONS

Age of Sperm Suspensions		1	2	3	4	5	6	7
		Concentration of the Sperm Suspensions						
		0.5%	0.2%	0.1%	0.05%	0.02%	0.01%	0.005%
Hours	Minutes	Hydrogen Potentials of Sperm Suspensions						
	5	7.70	7.80	7.85	7.88	7.90	7.95	7.98
	50	7.60	7.75	7.80	7.86	7.88	7.91	7.96
2	20	7.30	7.40	7.60	7.80	.....	7.88	7.92
4	20	7.26	7.30	7.53	7.70	7.87	7.87	7.91
6	20	7.20	7.37	7.45	7.56	7.86	7.88	7.95
17	20	7.26	7.40	7.50	7.74	7.90	7.98	8.04
20	50	7.20	7.40	7.50				
22	50	7.17	7.26	7.40	(7.74)	(7.88)	(7.91)	(7.98)
24	50	7.10	7.26	7.40				

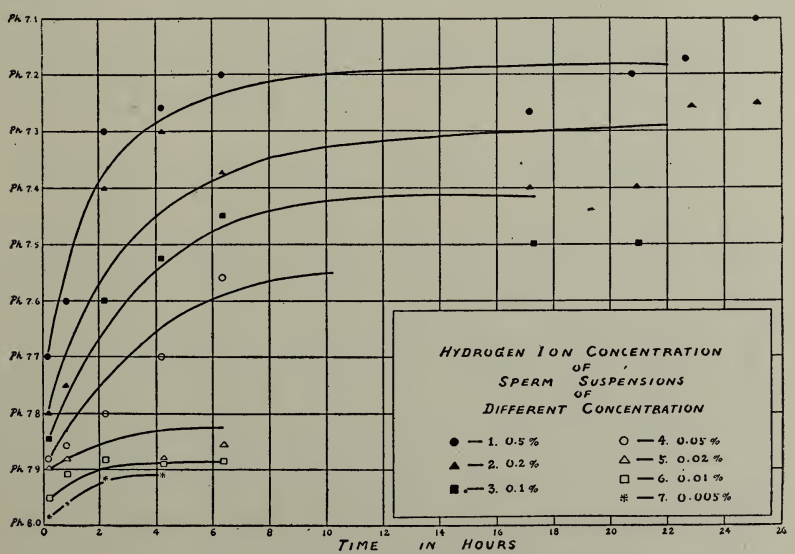


DIAGRAM I

the per cent of eggs that were fertilized by identical concentrations of sperm is given in Table III, and the results are shown graphically in Diagram II. The spermatozoa lived longest in

the most concentrated suspensions, as determined by the percentage of eggs fertilized by different concentrations of sperm.

TABLE III

LENGTH OF LIFE OF SPERM SUSPENSIONS

Age of Sperm Suspensions		1	2	3	4	5	6	7
		Concentration of the Sperm Suspensions						
		0.5%	0.2%	0.1%	0.05%	0.02%	0.01%	0.005%
Hours	Minutes	Percentage of Eggs That Were Fertilized in Sea Water by Identical Concentrations of Sperm						
4	20	98	98	85	94	54	34	6
6	20	99	97	43	52	6	4	0
10	20	100	100	2	31	0	4	7
17	20	100	100	5	0	0	0	0
		Approximate length of life of spermatozoa as computed from their failure longer to fertilize eggs of the same species						
		17	17	17	10	6	6	4

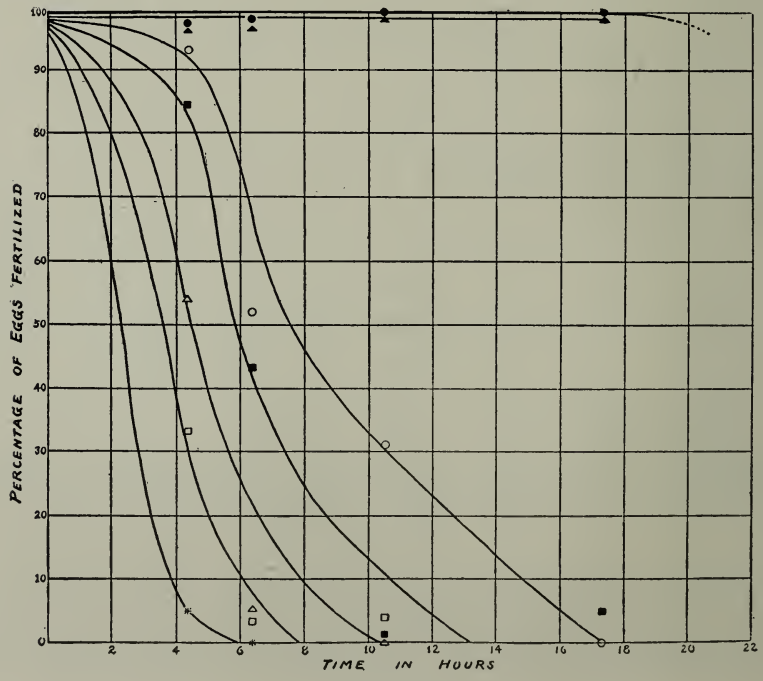


DIAGRAM II

The relative carbon dioxide production of sperm suspensions is shown in Table IV.

TABLE IV

Total Carbon Dioxide Production of Sperm Suspensions

Number	Sperm Concentration, Per cent	Calculation of the Relative Carbon Dioxide Production per Unit Concentration of Sperm	Approximate Length of Life of Spermatozoa
1	0.5	13	17+
2	0.2	18	17+
3	0.1	26	17+
4	0.05	37	10+
5	0.02	32	6+
6	0.01	45	6+
7	0.005	60	4+

It has also been shown by Cohn that spermatozoa live for the longest time when the carbon dioxide tension is about one millimeter. Increasing the carbon dioxide concentration increases the hydrogen ion concentration of the suspension, decreases the activity of the spermatozoa, and increases the length of their life.

The Action of External Agents on Spermatozoa

As early as 1856, Koelliker made a series of observations on the activity of the spermatozoa of several mammals. He found that the mature sperm from the vas deferens is quiescent, but can be aroused to activity by a number of substances. Best of all are the secretions of the glands that are connected with the vas deferens in the male. Next come salt solutions of various kinds. On the other hand, in pure water, the spermatozoa come quickly to rest. They are not immediately killed and can be activated if small amounts of salts are added.

The Hertwigs made many experiments in 1887 on the effects of drugs on the spermatozoa of sea-urchins. If sufficiently concentrated they bring the sperm to rest. Weak solutions of nicotine quiet the sperm in one and a half hours to such an extent that they do not fertilize the eggs, but these spermatozoa quickly recover their activity and their fertilizing power if returned to

fresh sea water. Chloral hydrate .5 per cent, brings the spermatozoa to rest in five minutes, but they recover in sea water. Quinine .05 per cent stops all movement in 35 minutes. Recovery takes place after 20 minutes in sea water. These drugs seem to produce their effects by quieting the sperm. Strychnine .01 per cent has no effect. The sperm will fertilize the egg in such a solution. Morphine also has no effect in weak solutions.

Inactive sperm in the testis may retain its fertilizing power for a long time. In the ducts and gonads of the male of many animals the sperm is as a rule at rest. In most insects the sperm is stored up in the receptacles of the females. Here also the spermatozoa are at rest, or may be only slightly active. In the honey-bee the sperm received in the nuptial flight may retain its fertilizing power for five or six years. In the bat, copulation takes place in the autumn, and fertilization occurs in the following spring after hibernation; but Hartman ('27) suggests that copulation may occur again in the spring and supply the functioning spermatozoa. The length of life of the human spermatozoa in the oviducts of the female has been supposed to extend over three weeks, but as Mall and others have shown, it is probable that they lose their power to fertilize much sooner than this, perhaps within forty-eight hours. Thus, even though their activity may last for a long time in the oviducts, their power to enter an egg does not last so long; but whether because of lessened activity, or because there is a loss of some essential substance in the tube is not known.

Steinach ('94) showed that extracts from the seminal vesicles of the male rat make the rat's sperm more active even than do salt solutions. He also showed that when these glands were removed and the males mated to females, fewer young were born, which he attributes to the inactivation of the sperm. The experiments are, however, not convincing. It should be recalled that it is not the activity of the sperm at the moment of ejaculation that would seem to be the essential factor but rather its later activity in the oviducts of the female which must be traversed in order to reach the upper end of the tube where the eggs are fertilized.

It has long been known that X-rays and radium sterilize animals for some time. Mature sperm will, after long exposure, lose altogether their power to become active, and will, after longer exposures, even disintegrate. Not only the mature sperm, but

also the sperm-producing cells of the testes are injured or destroyed by long, or rather by repeated exposure to X-rays, although other tissues of the same animal are not injured by the same exposure. How the action takes place is not definitely known, but X-rays and radium emanations appear to be almost specific agents for sperm cells; at least they are more quickly injured than the other cells of the animal.

It has been shown that the chemical composition of the medium in which the egg and spermatozoa are placed may have a direct influence on the chance of fertilization taking place. It has

TABLE V

THE EFFECT OF THE ADDITION OF KCN TO SEA WATER ON THE LENGTH OF LIFE OF THE SPERM SUSPENSION

Concentration of Suspension, Per Cent	Time of Insemination	Age of Sperm		Number of cc. of 0.1*N* KCN Added to a Liter of Sea Water								Number of Drops of Sperm Added to Eggs
				10	2.5	1.25	0.62	0.31	0.16	0.04	0	
		Hours	Minutes	Percentage of Eggs Fertilized When Sperm Added to Eggs in 5 cc. Sea Water								
0.04	11.05	0	0	99	97	....	97	....	93	97	71	8
	12.05	1	0	100	98	....	....	....	....	....	91	
	1.40	2	35	1	100	....	....	....	....	....	34	
	2.45	3	40	2	100	....	79	....	16	26	4	
	7.15	8	10	....	73							
0.7	3.45	0	0	....	....	....	....	....	....	....	....	1
	4.40	0	55	....	100	100	100	100	....	....	100	
	9.50	18	5	....	100	100	100	100	....	....	100	
	9.45	42	0	....	100	5	99	98	....	....	97	
	2.00	46	15	....	98	0	4	2	....	....	2	
	4.45	49	0	....	97	....	6	0	....	....	0	
	7.00	51	15	....	2	....	1					

been shown by Drzewina and Bohn ('12) that the addition of potassium cyanide to sea water prolongs the life of the spermatozoa. This has been confirmed and more accurately determined by Cohn (Table V). The results show that spermatozoa are quite inactive in those concentrations of potassium cyanide that are most effective in prolonging the life of the spermatozoa.

Lillie and Cohn have both pointed out that when the relative concentration of the egg-water and sperm is such that the activity of the sperm is decreased, the length of life of the spermatozoa, as measured by their ability subsequently to fertilize eggs, is greater than that of sperm exhibiting constant activity in sea water for an equal length of time.

It has been shown by Loeb ('14–'15) that spermatozoa of the sea-urchin will not fertilize eggs in a N/2 NaCl solution, or in any combination of NaCl, $MgCl_2$, and KCl, even in the concentrations in which these salts are found in sea water; but if $CaCl_2$ is added to such solutions, or to combinations of them, fertilization will take place. The addition of a small quantity of NaOH to salt solutions increases the fertilizing power of the sperm. R. S. Lillie ('16) and Gray ('13) have found for starfish sperm that increase in the acidity of sea water, by the addition of traces of HCl, decreases the fertilizing power of the sperm; but the addition of NaOH steadily increases the fertilizing power of the sperm up to a point where the alkali is strong enough to permit fertilization, but inhibits cleavage.

It has been shown both by Loeb and by F. R. Lillie that the activity of the sperm does not in itself insure fertilization. Loeb found that if ripe eggs of Arbacia are put into a M/2 NaCl solution and are then transferred to a neutral mixture of M/2 NaCl and $\frac{3}{8}$/M $MgCl_2$ in the proportion in which these salts exist in sea water, the eggs are not fertilized when sperm are added, although the spermatozoa become very active. If, however, to this solution one drop of a M/100 solution of NaOH or eight drops of a M/100 $NaHCO_3$ are added, most of the eggs will be fertilized. Again, if eggs are placed in a neutral mixture of NaCl plus KCl, they are not fertilized, although the sperm may be so active that the eggs are rolled around by the sperms sticking to their membrane. If a little sea water is added to the mixture, the eggs are instantly fertilized by the sperm that are present. The sea water brings in Na and Ca. If NaOH is added to the mixture, and fertilization occurs at all, only a few eggs are fertilized, but if $CaCl_2$ is added many of the eggs are fertilized. If both are added all the eggs are fertilized. The lack of $CaCl_2$ and NaOH acts as a block to the entrance of the sperm, which is removed when they are added. Loeb thinks that the block is due to a change in the physical condition of the surface of the egg.

The spermatozoa of Nereis are brought to rest by the addition of CO_2 to the sea water; even one per cent of CO_2 suffices. Normal activity is shown again when the amount of CO_2 is decreased below this. Arbacia sperm is active in a 2.5 per cent CO_2-solution; Chaetopterus sperm in a 20 per cent. Cohn ('18) has shown that the fertilizing power of the sperm of Arbacia is lost after an interval inversely proportional to the initial concentrations of the sperm suspensions. He interprets this result to mean that

Fig. 1.—Aggregation of sperm of Nereis by egg-water. (After F. R. Lillie.)

the sperm is less active in the stronger concentration owing to the greater amount of CO_2 present, resulting from their own activity. More CO_2 being produced in the more concentrated solutions, the spermatozoa remain alive longer because they are kept quieter by their own by-product, viz.: the CO_2 produced by them.

If fresh sperm of Nereis is put into the sea water aggregations of spermatozoa are quickly formed. The water may temporarily assume a flaky appearance (Fig. 1). In the interior of each aggregate the sperms are at rest; those at the periphery are still active and cause the mass to rotate in the water. The aggregates increase in size by the addition of more spermatozoa to the sur-

face of the mass, while those formerly at the periphery come to rest within the mass. This phenomenon is due, according to Cohn, to the rapid production of CO_2 by the spermatozoa. "Any aggregate of greater concentration of spermatozoa, by producing more CO_2, becomes a center of aggregation which, when once begun, is bound to proceed to the limit on account of increased CO_2 production." If the water be made hyperalkaline the aggregation does not take place, because the alkali will neutralize the CO_2 as rapidly as it is formed. Cohn has suggested that the aggregations are due to the inactivity of the sperm caused locally wherever the amount of CO_2 is sufficient to make a few spermatozoa less active. As more sperm run into this region, they also are made less active, and their presence there adds further to the amount of CO_2 present. Lillie raises an objection to this interpretation on the ground that the sperm at the periphery of the aggregate are quite active, and also that their movements are those of rotation such as shown by them in their thigmotactic (contact) response. Neither of these objections is fatal to Cohn's interpretation; for it seems probable that the new spermatozoa that reach the periphery of the aggregate may for a time retain a part at least of their former activity. As they slow down new spermatozoa come on to take their places. That their activity may be rotatory rather than spiral is explicable by their contact with the more solid mass. Lillie thinks that the aggregations are better explained by the assumption of chemotaxis. The diffusion of CO_2 from the aggregate acts as a "gradient" to which the sperm react by changing their course towards the more concentrated regions. It is, however, not obvious that the results call for any such orienting reaction. The immense number of sperm present, at first swimming in all directions, may sufficiently account for the additions to the aggregates as soon as they are formed by the accidental meeting and sticking together of two or more spermatozoa.

Buller ('02) observed that when a bubble of oxygen is introduced into a suspension of Echinus sperm, the spermatozoa in the vicinity of the bubble remain active after those in the rest of the suspension have come to rest for want of oxygen. Between the active and the quiescent sperm there is a zone relatively free from sperm. Those active sperm that happen to swim nearer this outer zone (presumably by random movements) collect at its edge—i.e., on the side with relatively more CO_2. Here they come

to rest forming a ring of quiescent sperm. Lillie ('13) later made the converse experiment by introducing a drop of sea water charged with CO_2 into a sperm-suspension. A dense ring of quiescent sperm forms around the drop that increases in thickness as more sperm are added to the ring. The sperm at the periphery of the ring are for a time at least very active. Here, also, Lillie thinks a CO_2 gradient causes the sperm to turn towards the drop, but it would appear that a sufficient number of free swimming sperm is present in the sea water to account for the additions to the drop; for all sperm whose path happens to be in the direction of the drop will run into it and sooner or later be brought to rest. Unless it could be shown that not enough sperm enter by chance the region of the drop there seems to be no necessity for postulating an orienting reaction of the sperm to the CO_2 gradient, especially in the light of the fact that no such direct tropism has ever been demonstrated for spermatozoa, while direct observations have shown that no such orientation of spermatozoa really occurs.

There is a further result described by Lillie, which he thinks goes to show that the ring-formation cannot be explained by the inactivation of the sperm by the CO_2. Alcohol paralyzes the spermatozoa of Nereis in a 5 per cent solution and decreases their activity even in a 2 per cent solution. It does not cause ring-formation when a drop is placed in a suspension of sperm. Therefore, Lillie thinks that in addition to the inactivation in the CO_2 reaction, it is necessary to assume an orienting action on the sperm. But it is evident that if the drop of alcohol is strong enough and does not diffuse away too rapidly, it *must* cause a ring of sperm around or in the drop if it inactivates the sperm; for enough sperm must be present in such a solution as this for many of them to swim by chance towards such a drop. If, then, as stated, no ring forms, its absence must be due to other causes not explained. Its absence calls for explanation, and until this is found the result cannot be safely used as evidence against Cohn's hypothesis of ring formation in CO_2. The presence of a clear zone outside of the dense ring does not seem clearly explained by the assumption of a CO_2 gradient. Possibly the fact that all sperm that touch the dense ring become quiescent and that there is no return in the opposite direction may explain the relative scarcity of sperm in the clear ring in comparison with those in the suspension outside.

CHAPTER IV

AGGLUTINATION AND FERTILIZATION

When sperm is added to eggs of the sea-urchin that have been standing in a small amount of sea water, or to the sea water itself in which eggs have been standing ("egg-water"), an almost instantaneous formation of clusters of spermatozoa takes place that may last for only a few seconds, or for a minute or more (Fig. 1). The clusters then break up, and the sperm are still active and are capable of fertilizing the eggs. Buller who was the first to observe this instantaneous cluster-formation interpreted it as due to the presence of small particles of egg-jelly to which the sperm stick. Loeb ('14) later expressed himself in favor of the same view, but Lillie and Just have disproven this interpretation.[1] It is customary at present to speak of the sudden union of spermatozoa to form clusters as agglutination or iso-agglutination, and von Dungern ('02) who was the first, I think, to apply the term agglutination to the cluster-formation of sea-urchins' sperm, used the word in the technical sense as applied to cells sticking together when those of one species are introduced into the body fluids of another species. He speaks of the agglutination of the spermatoza of the sea-urchin on coming in contact with the jelly of the egg of the starfish. He found no substance produced by eggs that attracts the sperms through

[1] Loeb suggested that fertilizin is present only in the jelly of the California sea-urchin's egg, and is removed when the jelly is removed, as can be done by dissolving the jelly in weak hydrochloric acid. Such eggs may still be fertilized, but both Lillie and Just insist that in Arbacia, and in the sand-dollar, the eggs, after removing all of their jelly either by shaking or by hydrochloric acid, continue to produce fertilizin. Their experiments appear to give positive evidence in favor of this view. An escape from their conclusion might be to assume that, after shaking, the particles of jelly left in the fluid, or carried down by the eggs, would suffice to give the fertilizin that they find present. As to the HCl-treatment it would be necessary to suppose that the acid had not removed all the jelly.

a measurable distance, and states that the egg-water of Arbacia in capillary tubes does not attract the sperms of the same species.

Schücking ('03) observed an agglutination of the sperm of the sea-urchin in a very dilute solution of materials from eggs that had been disintegrated and dissolved in sea water. The eggs and jelly were ground in distilled water and after twelve hours the mass was boiled and filtered. The filtrate is acid. If a trace of NaCl is added, the concentrated solution injures and then kills the sperm. Diluted it excites and agglutinates the sperm. Still further diluted, it excites but does not kill the sperm. He observed cluster-formation that lasted for a short time and is not restored after separation of the spermatozoa. If the filtrate is dialized, then boiled, the dialized part is still acid and activates the spermatozoa but does not agglutinate them. The non-dializable material, also acid, agglutinates and injures the sperm. It contains crystals like those that have been obtained from human semen.

When the concentrated, non-dialized substance is put into a capillary tube which is then placed in sea water containing sperm, a white zone of injured sperm is formed at the outlet, then a zone of agglutinated sperm, still active, and lastly a zone of very active sperm. If the fluid in the capillary tube is much diluted, the agglutination does not take place, and sperms collect in large numbers in the outer zone and are very active there. Schücking concluded that the quieting of spermatozoa, that have remained a long time at the periphery of the egg, is due to the acid of the egg or of its jelly. The spermatozoa are more active in a neutral or in a slightly alkaline medium. Owing to this relation Schücking attempts to explain a result that he observed, viz., that when a small amount of sperm is added it takes longer to fertilize the eggs than when more sperm is added. In the first case the acid of the jelly agglutinates the first spermatozoa that reach it, causing them to rotate on the surface, while in the second case when more sperm is added, the alkalinity of the sperm neutralizes the acid of the jelly and the spermatozoa enter it radially. Hence, better and quicker fertilization occurs with more sperm. However, it is obvious, I think, since there is a better chance for one or more sperm to strike the egg-jelly in a radial position when more are present than when only a few are swimming at random, that Schücking's suggestion is of doubtful value.

Schücking reports that when the sperm of sea-urchins have lost their activity, after six to eight hours in sea water, they will not fertilize the egg, even of the same species, but if the combination of eggs and sperm is gently rubbed, the sperm may then fertilize the eggs. Furthermore he states that centrifuged sperm that have lost their tails, if rubbed together with eggs, will fertilize the eggs, but will not do so if such sperm are simply brought in contact with the eggs. The result is interpreted to mean, that by rubbing, the heads of the sperm are brought into such close contact with the egg that they are taken in; but another interpretation is perhaps more plausible. By rubbing the eggs some activating substance may be set free, and then, if any intact sperms are present, this substance may call forth enough activity to cause some of them to fertilize a few of the eggs in a normal manner. It may, of course, be true that, after rubbing the eggs, the jelly is so far removed, or reduced in amount, that the sperm-heads come into close enough contact with the egg surface to call forth the fertilization reaction. This is probably what Schücking had in mind, but it may seem doubtful whether such union would take place unless the tip of the sperm penetrated the surface of the egg.

De Meyer ('11) found that if a drop of sperm is put in a concentrated egg-water solution there takes place at once an agglutination of the spermatozoa. They lose their motility, and form a very irregular network. In a less concentrated solution the sperm become motile, but less active than in sea water. A dilute solution arouses them to activity, when neutral or very nearly so. Capillary tubes (.5 to 0.75 in diameter) were filled, some with sea water, some with egg-extract (the tubes closed at one end with paraffin), and were plunged into sea water containing an abundance of sperm, and left there from one to three hours. The spermatozoa did not enter the tubes with sea water, while those with egg-extract contained a plug of sperm. On this quite insufficient evidence De Meyer concludes that the egg-extract has a chemotactic influence on the spermatozoa. To test this further he mixed several drops of sperm with egg-extract, leaving them together for fifteen to twenty minutes. The extract was not sufficiently concentrated to agglutinate the sperm. Eggs were then added to the mixture. The sperm did not then concentrate around the eggs, yet they still possessed the power to fertilize the eggs since membrane formation took place. De Meyer concludes from

this evidence that the egg-extract has "annihilated" the chemotaxis of the spermatozoa, but has left intact the contact-reaction necessary for fertilization. That a reversal of the slight agglutination may occur to a sufficient degree to permit some sperm to fertilize the egg is not taken into consideration. Later observers have shown in fact, that reversal does occur if the egg-water is not too strong. De Meyer's evidence, then, cannot be considered adequate to show that chemotaxis takes place. The observations can be accounted for on other grounds. In one respect, however, it raises a new and important question, namely, whether if sperm have been once fully agglutinated they can subsequently penetrate the jelly and fertilize the egg.

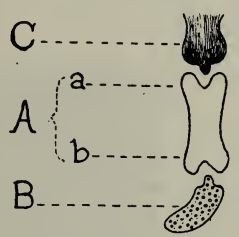


Fig. 2.—Amboceptor (*A*) with complement (*C*) and blood corpuscle (*B*), illustrating Godlewski's hypothesis of fertilization. (After Godlewski.)

In 1911 Godlewski made a carefully guarded comparison between the results of the antagonistic action of sperm of different species and serological work on blood. He drew attention particularly to Erlich's side chain theory (Fig. 2), in which an amboceptor (A) may unite at one end with a red blood corpuscle, and the other with the "complement" (C). He pointed out that the "analogy" may have value if, as Loeb supposed, the first step in development involves cytolysis of the surface of the egg.

An extensive study of the agglutination of the sperm by egg-water has been carried out by F. R. Lillie ('13, '14, '15), who has confirmed the earlier observations that normally a substance is set free from the eggs of the sea-urchin that calls forth this reaction. The activity of the spermatozoa is at first greatly increased in egg-water and they immediately become agglutinated ("cluster-formation"). Lillie also found that the clusters quickly break up in weaker egg-water. Agglutination is spontaneously reversible, while aggregations formed by CO_2 and other substances are not reversible. Lillie makes a sharp distinction between agglutination and aggregation because the former, he states, is reversible, and the latter is not. Aggregation may also be distinguished from agglutination by means of certain additional tests. Concentrated egg-water may completely immobilize the spermatozoa. They may then become unable to fertilize the eggs, and this is due, Lillie thinks, to the aggregation of the sperm rather than to

agglutination. Nevertheless, it is not entirely clear that agglutination caused by concentrated egg-water may not be essentially the same process as aggregation produced by other substances. Some of Lillie's observations in support of his view may now be stated in more detail.

According to Lillie the agglutinization is best shown as follows. A drop of sea water containing active sperm is placed on a slide. A cover-slip, supported by glass rods, is laid over this "sperm suspension." By means of a capillary pipette, a drop (2 to 4 mm. in diameter) of the egg-water is next introduced between the slide and the cover-slip. At once a very violent reac-

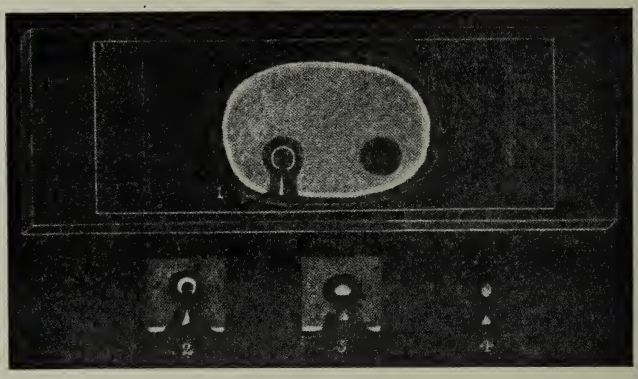


Fig. 3.—Ring-formation of sperms of Nereis caused by introducing a drop of sea water containing CO_2 into a sperm-suspension. (After Lillie.)

tion follows. In the first second, the spermatozoa within the drop are aroused to great activity, and form small agglutinated masses which persist for a period of three to five seconds. After this no further fusion of the masses takes place. "While this has been going on in the interior of the drop, a ring has formed at its periphery, and a clear zone arises external to it. The ring is at first continuous, but it ruptures in numerous places in two or three seconds and each segment contracts quickly to an agglutinated mass." The agglutination disappears after a few seconds to a few minutes, and the sperms have become relatively immobile. The preceding account applies to Arbacia and to Nereis. It has also been observed in other sea-urchins. Three kinds of effects are involved according to Lillie: (1) *activation*, (2) *aggregation*, (3) *agglutination*. The increased activity has not been observed

in all animals examined. The aggregation is similar to that formed around a drop of CO_2 (Fig. 3), and may possibly be due to the same or similar causes. It may be eliminated by adding an alkali to the sperm suspensions.

When agglutination occurs, the spermatozoa are stuck together and cannot, while agglutinated, be shaken apart, but the reaction is spontaneously reversible. As shown by Loeb, agglutination does not occur if the sperms are first immobilized by KCN. It appears, therefore, that impact of the sperm is necessary to make them stick to one another.

The agglutinating substance, fertilizin, is produced only by mature eggs. It is not present in the blood or tissues of the animal. It may continue to be produced for some time (two or three days even) after the eggs are washed. The jelly of the egg contains it as long as it is secreted by the egg. As soon as the eggs are fertilized it ceases to be produced and if the jelly is removed at this time no more of it can be obtained from eggs in sea water.

The agglutination affects only the heads of the spermatozoa. The tails are unaffected. The agglutinating substance is non-dialyzable; it will not pass through a Berkefeld filter, but will pass through hardened filter paper. It is very heat resistant, being destroyed only slowly by being boiled. It may be kept in sea water for several months, but slowly disappears. Lillie concludes that it is colloidal, but Glaser ('14) has shown that it does not give the usual protein tests. Its efficiency (concentration) can be measured by the number of seconds that the sperms remain agglutinated. According to Richards and Woodward ('16) the action of the substance is accelerated at first when acted upon by X-rays (about two minutes' exposure), non-effective after five minutes' exposure, and inhibited after a longer exposure. "It thus possesses some ferment analogies" (Lillie). If a sperm suspension is once agglutinated completely, it cannot be again agglutinated by adding more of the agglutinating substance.

It might seem possible to determine whether the presence of fertilizin in the egg is really necessary for the union of the egg and sperm by removing the jelly, and, then, when all the fertilizin had been set free from the egg, attempt to fertilize it. If the sperm should fail to fertilize, it might seem probable that the presence of fertilizin *in the egg* is really essential to the union.

Experiments have been made by Lillie to test this point. He finds that when the eggs have ceased to produce fertilizin they cannot be fertilized, but since they cease to produce fertilizin only after washing for two or three days, the results are open to the objection that the eggs by this time have undergone changes in the sea water such that their failure is not due to lack of fertilizin but to their dying condition. Loeb suggested, in fact, that this is the cause of the failure in question.

It might seem possible to demonstrate that the failure to fertilize old eggs is not due to the condition of the eggs, but to the absence of fertilizin in them, by placing such old eggs in fresh egg-water, and then adding fresh sperm. If a spermatozoön should then enter an egg, and cause it to develop, it would appear either that the presence of fertilizin outside the egg is necessary, or that some of it has been absorbed by the egg and has made the entrance of the sperm possible. But the conditions apparently are not so simple as this, as other evidence seems to show. Woodward ('18), who carried out such an experiment, reported that the eggs of Asterias and Arbacia that have been washed so that they no longer produce fertilizin, can be fertilized if placed in egg-water (containing fertilizin) to which sperm is also added. This result seems to supply the crucial evidence, showing that fertilizin is essential to fertilization even though all of it at first present in the egg has been set free, but unfortunately Miss Woodward's results have not been confirmed. Lillie and Just ('24) question this result and suggest that the "added secretion acts by modification of the pH of the medium."

Lillie points out that the agglutination of the sperm by the egg might be regarded as necessary to bring about the intimate union essential to the further action of the spermatozoön, or it might be supposed that the fertilizin is the substance that activates the egg when combined with a spermatozoön (or something supplied by the latter) and that the sperm brings this back into the egg. He argues in favor of the latter view because spermatozoa may actually enter immature eggs devoid of agglutinating substance, but then exert no fertilizing action. The validity of the argument may be questioned because other conditions are present in the immature eggs and no effect of the sperm on them is to be expected. C. R. Moore ('17) has found that eggs treated with butyric acid for the optimum time to produce artificial parthen-

ogenesis which frees them from agglutinating substances may be entered by sperm after removal of their membrane. These sperm remain perfectly inert in the egg protoplasm. The only conclusion to be drawn from such a result is, in my opinion, that the fertilizin is not essential to the entrance of the spermatozoa. The failure of the spermatozoa to produce any effect in such eggs would seem more easily explained as due to progressive changes already induced by the butyric acid, rather than that the sperm in general "exerts no fertilizing action whatever."

Lillie concludes that, "The conception of the mechanism of fertilization resulting from these considerations would thus be that a substance borne by the egg (fertilizin) exerts two kinds of action, (1) an agglutinating action on the spermatozoön and, (2) an activating action on the egg. In other words, the spermatozoön is concerned, by means of a substance which it bears and which enters into union with the fertilizin of the egg, to release the activity of the substance within the egg."

The cessation of production of fertilizin when the egg is fertilized, that was discovered by Lillie and confirmed by Moore, Just, and Woodward, is the basis of Lillie's argument that fertilizin itself is the cause of the activation of the egg. He points out that if unfertilized eggs are first deprived of their membranes and then cytolyzed "thus extracting the interior substances," the extract has a powerful agglutinating effect on the sperm.

But if eggs are repeatedly washed for 48 hours until their production of fertilizin is very much reduced, and are then shaken to pieces in the sea water containing fertilizin which they themselves have secreted, the fertilizin is neutralized by other substances set free from the interior of the egg. He concludes that "eggs contain in their interior a substance capable of combining with the agglutinating group of the fertilizin, but which is separate from it as long as the egg is inactive; this substance I called antifertilizin." He suggests that the fertilizin introduced by a spermatozoön is responsible for this union within the egg, hence a fertilized egg no longer produces fertilizin. This union is interpreted, apparently, as the essential factor that starts the development. It would seem to follow that, in order to produce its effect, the fertilizin must first combine with something in the sperm (activating its ovophile group), which then entering the egg brings about some sort of union between the antifertilizin of the

egg and the rest of the fertilizin still present in the egg. When this is accomplished development begins.

Lillie's hypothesis may be summed up in the following quotation. "The essential conclusion is that fertilization is a reaction between three bodies of which one is borne by the sperm and one by the egg; the third body which is secreted by the egg reacts with both the others. The spermatozoön functions essentially as an activator of the third body which I propose to name 'fertilizin' (sperm isoagglutinin). The latter when activated enters into certain reactions in the cortex of the egg which lead to membrane formation. In order to give a concrete working conception, I have pictured the fertilizin as possessing two side-chains active in fertilization, viz., one reacting with the sperm which I call the 'spermaphile side-chain' or group, and the other reacting with the egg which I call the 'ovophile side-chain' or group. The chemical group of the sperm, which reacts with the fertilizin, is named the sperm-receptor and that of the egg the egg-receptor. We are thus furnished with a concrete conception which answers very well for the purposes of description; it need not, however, be taken too literally. The terminology has been adopted largely from immunology because it seemed best suited to express the facts. The terminology and the theory are significant only to the extent that they give a brief description of the facts, and serve as a working hypothesis."

The details of this pictorial representation of Lillie's conclusions can be best understood by the diagram (Fig. 4) in which they are symbolized.

In the first sector (below, numbered 1) is shown the imaginary arrangement of the "substances" in the unfertilized egg and in the spermatozoön. The cortex contains the fertilizin of which three bodies are represented each with a spermophile and an ovophile combining group. The sperm has a receptor to fit the spermophile group at one end of the fertilizin; the egg also contains receptors capable of filling the ovophile group of the fertilizin. Alternating with the egg receptors are found antifertilizin molecules capable of uniting with the spermophile group of the fertilizin.

In the second sector one sperm has united with one fertilizin molecule at the surface and at the moment when this occurs the ovophile group at the other end of the fertilizin molecule is

closed by an egg receptor. Then all the remaining fertilizin molecules become occupied at one end of each by anti-fertilizin (in the egg) and at the other end by an egg-receptor. All the fertilizin being "occupied" no more sperm can enter. This contagion extending from the point of penetration of the sperm may be compared by analogy to the spread of a stimulus.

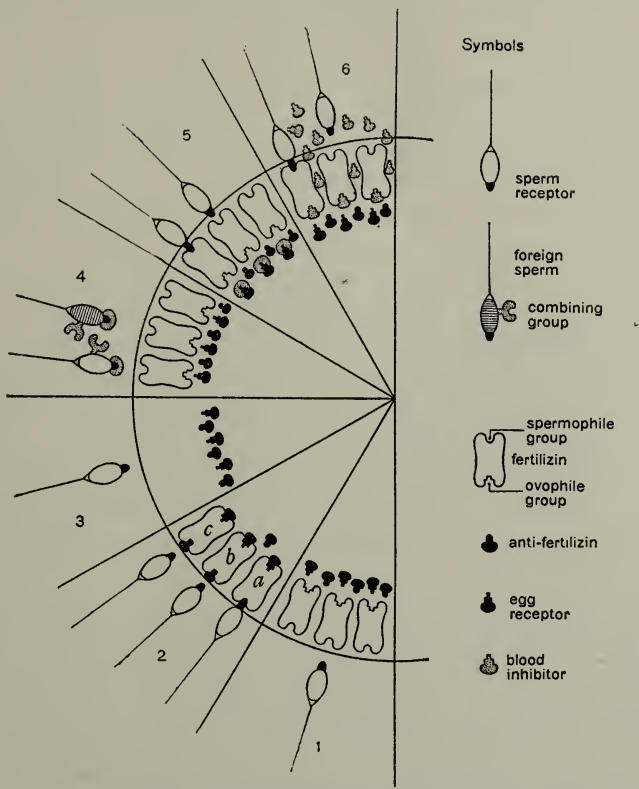


FIG. 4.—Scheme illustrating Lillie's theory of fertilization. (After Lillie.)

Lillie points out that this representation admits of five blocks in the process of fertilization that are illustrated in the different sectors; (1) absence of fertilizin (sector 3); (2) occupancy of the sperm receptors (sector 4); (3) occupancy of the egg-receptors (sector 5); (4) occupancy of the ovophile side-chain of the fertilizin (sector 6); (5) occupancy of the sphermophile side-chain (sector 2; b and c). Facts that find their representation are in (1), long-washed eggs, in (4) the inhibition by the

blood, in (5) non-fertilizable condition of the eggs already fertilized. The other two methods have not been found.

Godlewski ('26) has recently made the following criticism of Lillie's fertilization hypothesis. He says that the "hypothesis gives the impression of a schematic picture, or of an imagined conception of the phenomenon of fertilization, rather than furnishing a real explanation of the facts of development. The hypothesis is characterized primarily by its speculative nature, and before accepting it a good deal of proof would be necessary." "Is it true, for instance, that the substance fertilizin has the properties of an amboceptor? Without insisting on the circumstance that the author has not succeeded in isolating fertilizin, but, following the methods used sometimes to support serological hypotheses, he infers its existence from the effects it produces, one looks in vain for positive proofs that authorize him to consider the fertilizin as having the structure of an amboceptor." "I find in Lillie's hypothesis a series of statements about which we can only say that they might be correct without being able to affirm that the process takes place in reality. The application of the hypothesis to sperm antagonism seems very doubtful." Godlewski raises a further question, that Loeb had already called attention to, as to whether it is justifiable to use the serological term *agglutination* (in contrast to aggregation) to describe the immediate and reversible action of the egg-water on the sperm. Such action does not correspond exactly with the application of the term agglutination in bacteriology. In fact, bacteriologists admit agglutination of immobile as well as of dead bacteria. If the mobility of the sperm is a necessary condition of agglutination, the phenomenon observed is different from that of serology. Lillie holds that the reversibility of the agglutination process in the sperm is one of its chief characteristics. While it is true that, by artificial means it is possible to disagglutinate under certain conditions, yet without such intervention true agglutination is irreversible and not transitory.

Lillie's results relating to the production of fertilizin have been confirmed by Just on the egg of the sand-dollar, Echinarachnius parma. Here, also, water charged with fertilizin agglutinates sperm-suspensions. Only ripe eggs give off the fertilizin. It can be more quickly removed by repeated washings from the eggs of the sand-dollar than from the eggs of Arbacia. Eggs, freed from

their jelly that is "loaded with fertilizin" by gentle shaking, continue to set free fertilizin. The jelly may also be removed by the addition of a little HCl to sea water. Eggs freed from their membranes in this way also continue to set free fertilizin, and eggs from both sets can be fertilized as long as fertilizin comes off. Rarely a female is found whose eggs are without jelly. These eggs also secrete fertilizin and can be fertilized.[2] The following table gives the results from the eggs of three females of Echinarachnius washed seven times between 8:30 and 12:10 P.M. The percentage of eggs that cleaved, i. e., eggs that were fertilized (last column) steadily declines. Fresh sperm was used for each insemination.

TABLE VI

Time	cc. Sea Water Used	Time in Seconds	Dilution	Cleavage, Per Cent
8.30	100	8	1/1600	90
9.00	200	8	1/100	90
9.15	100	6	1/800	85
9.40	200	6	1/400	80
10.00	200	6	1/200	70
12.00	75	4	1/50	10
12.10	85	4	.....	10

A statement made by Lillie ('13) and confirmed by Just ('15) may be significant for the interpretation of the action of fertilizin. Lillie found that if the unfertilized eggs of Nereis are washed once, usually no more agglutinin can be demonstrated in the egg-water from such eggs, but the eggs can still be fertilized. They then give off immediately a large amount of fertilizin. Just also states that if "the eggs be washed by changing the water two or three times the fertilizin is no longer secreted in detectable amounts,

[2] One curious result is recorded by Just. Eggs were obtained from one female that appeared to be exceptionally good, but few of them could be fertilized. Another set of eggs that behaved similarly were tested for fertilizin and were found to be producing it. They were washed and after 90 minutes were tested. Very few were fertilized. But when a little ether was added to the sea water 90 per cent of the eggs cleaved. Just interprets the result to mean that failure in the first instance to fertilize was not due to loss of fertilizing power as a result of the washing, but to some condition of the membrane that was removed by the ether.

i. e., there is not enough to agglutinate the sperm. Such eggs are none the less fertilizable by sperm, giving off at the time of insemination more fertilizin. . . ." Two facts stand out conspicuously in these statements. First that fertilization is possible in the absence of detectable free fertilizin. This may appear to mean that the presence of fertilizin outside the egg may not be essential to fertilization. Lillie himself has explicitly stated that the agglutination test of the sperm is not a sufficient test of the presence of fertilizin. This concession removes the possibility of finding out whether an egg cannot be fertilized when it ceases to set free fertilizin, for there is no longer a way of determining when it has ceased. The outpouring of fertilizin from the egg of Nereis at the moment of insemination does not seem consistent with the view that the essential element in fertilization is the locking up of the fertilizin in the egg by the antifertilizin. The phenomenon appears rather incidental than essential in the light of these results.

An interpretation of the aggregation of spermatozoa different from that heretofore considered has been offered by Gray ('15). As is known spermatozoa of sea-urchins become quiescent in a slightly acid medium. By neutralizing the solution, and then making it alkaline, the sperm become active again. This result suggests he thinks that the movement of the spermatozoa is dependent upon electrical properties of the cell and of its medium. The two following experiments are given in support of such a view:

1. Sperm are suspended in a neutral isotonic cane sugar solution. Their activity ceases, but if a trace of alkali is added they become motile. When an electric current is passed through such a neutral solution the sperm "travel" rapidly to the positive pole where they accumulate. Around the negative pole, however, the sperm become very active, due no doubt to the liberation of alkali whose presence can be shown by an indicator. If the sperm are suspended in a faintly acid solution of cane sugar, no migration takes place to the positive pole and no activation is observed at the negative pole. In this solution the electric current causes the sperm to form a net-like aggregation throughout the solution. The facts suggest that the motile sperms carry a negative charge on their surface which is lost in the presence of free hydrogen ions.

2. The action of trivalent cations is shown by adding a

drop or two of a weak solution of cerous chloride to a sperm-suspension of Arbacia. The sperm become intensely active, and rapidly aggregate in clumps. The sperm in the clusters are at first extremely active, but soon become motionless. The effect of the trivalent ions is removed by the addition of sodium citrate. These results are in favor of the view, he suggests, that the trivalent ions affect the sperm by virtue of their electric charge.

When a drop of cerous chloride or neodymium nitrate is injected into a sperm-suspension under a cover glass, ring formation, etc., takes place that is identical to all appearance with that described by Lillie for egg-extractives. "It would appear that the behavior of the spermatozoa towards 'agglutinin' is identical with its behavior towards a trivalent cation." If this is established the phenomenon of agglutination may be a physical phenomenon connected with the electric charge of the spermatozoa. No evidence is given that this agglutination may not be only an aggregation, but the relation of agglutination to aggregation has not been as yet made clear. The latter is said to be reversible but strong solutions of egg-water appear to aggregate the sperm as well as to agglutinate them.

It has been shown by Richards and Woodward ('15) that after radiating egg-water (with X-rays), some substances in it derived from the egg are acted upon by X-rays in somewhat the same way as are enzymes. If this substance is identified as the lipolysin, obtained from eggs, that has been shown to affect the hydrolysis of higher fats, the question remains to be determined whether it plays its rôle in the initiation of development by being carried back into the egg with the sperm (as on Lillie's view regarding the ambocepter), or whether it acts on the egg itself, being locked up there, so to speak, by the union with the anti-fertilizin remaining in the egg. The fact that there is another substance present in the egg-water that may act as a parthenogenetic agent, may or may not have a bearing on this question; for, there are many other agents that have this effect that can not possibly be contained in the egg or in the egg-water.

Glaser ('14) subjected the egg-water from Arbacia eggs to a series of physiological tests which show that if proteins are present their concentration is too low to give the usual reaction. If minute traces of protein are present they may come from the egg-jelly. An attempt to isolate the active agents of egg-water

was made by Miss Woodward ('18). The Arbacia secretion when saturated with $(NH_4)_2SO_4$ gave a white precipitate which, on being purified by dialysis, was found to have an agglutinative property on the sperm. By a different method another substance was obtained that does not agglutinate the sperm, but has a parthenogenetic action on unfertilized eggs. This second substance was called lipolysin, and Woodward ('15) and Glaser ('21, '22) have shown that it accelerates the hydrolysis of fats. Whether both of these substances are involved as separate molecules in fertilization, rather than as one body (the amboceptor, postulated by Lillie), or whether the amboceptor, so called, has here become separated into its ovophile and spermophile parts, is not, as they point out, apparent from this analysis.

The Rôle of Fertilizin in Cross-Fertilization

Lillie ('13) has shown that egg-water and also the coelomic fluid of Arbacia agglutinates the sperm of Nereis. The effect of the former, therefore, may not be due to the fertilizin but to another substance in the eggs. Egg-water from Nereis eggs does not agglutinate the sperms of Arbacia. Since cross-fertilization does not occur here the results tell us nothing further of the rôle of fertilizin. The problem has, however, in another combination, been studied by Just ('19), namely in a cross between the sand-dollar (Echinarachnius) and the sea-urchin (Arbacia) where cross-fertilization takes place. He found that the egg-water from Echinarachnius egg does not agglutinate Arbacia sperm yet the eggs may be readily cross-fertilized by Arbacia sperm. It is significant, perhaps, that the Arbacia sperm are made intensely active in this egg-water from Echinarachnius eggs. Just states "In no other case that I know is the activation so marked." In this connection it is not without interest that egg-water from Arbacia eggs agglutinates the sperm of Echinarachnius, yet cross-fertilization is here extremely difficult. The agglutination of the sperm is stated to be "reversed" in a few seconds after its occurrence. It is observed only in relatively concentrated solutions of egg-water. Just has shown that the agglutination is probably not due in this case to fertilizin but to some other substance set free from the egg. This evidence, while interesting in itself, does not, in the light of the other cross, show any

evidence of the failure of this combination to produce cross-fertilization. The reverse cross was successful in the absence of fertilizin agglutination.

Experiments have also been made with the two common sea-urchins of the Pacific Coast (Strongylocentrotus franciscanus and S. purpuratus) by Loeb ('14, '15) and by Lillie ('21). Loeb found that the egg-water of franciscanus does not produce "cluster formation" of the sperm of purpuratus, yet cross-fertilization is easily brought about. Lillie states that there is not much difference in the relative ease with which crossing may be brought about between the two species. Lillie confirmed Loeb's result on the absence of agglutinating power of the egg-water of franciscanus on the sperm of purpuratus. In the reciprocal combination, namely, the egg-water of purpuratus, he found that in the majority of cases an apparent agglutination of the sperm of franciscanus occurs. Cross-fertilization of such eggs is known to occur. Lillie concludes from these and other observations that in this case it is a different active principle from fertilizin that causes the cross-agglutination, and adds: "One cannot therefore argue from the absence of the agglutination reaction in any given case that there is no connection between the modification of the spermatozoön evidenced by agglutination and fertilization. This is admittedly a serious limitation of the method for analysis of fertilization, but the limitation does not weaken the force of the positive results."

Lillie says further: "The adhesion of spermatozoa to one another is no part of the process of fertilization; indeed such adhesion could not occur in the sperm concentrations ordinarily used in fertilization or occurring in nature owing to the wide dispersion of the spermatozoa. It is the change in the individual spermatozoön, and the postulated reciprocal change in the fertilizin that is significant."

Lillie ('21) sums up the evidence which he believes covers the conditions of fertilization as follows: "(1) The production of fertilizin by eggs ceases at fertilization; correspondingly the eggs are 'immune' to spermatozoa. (2) The production of fertilizin by eggs ceases after membrane formation by butyric acid; correspondingly these eggs are not fertilizable. (3) If the fertilizin content of eggs be reduced by repeated washings the fertilization capacity decreases correspondingly. (4) The production of fer-

tilizin by eggs of the sea-urchin does not begin until after maturation; correspondingly the fully grown ovocytes with intact germinal vesicle are immune to spermatozoa, or, if spermatozoa enter such eggs, they are entirely without effect."

Antagonistic Action of Sperm of Different Species

Godlewski ('11) found that if the sperm of the annelid, Chaetopterus, is mixed with the sperm of the sea-urchin Sphaerechinus and allowed to stand from ten to fifteen minutes the mixture fails to fertilize the eggs of Sphaerechinus. Similarly the mixture of the sperm of the mollusc Dentalium and Sphaerechinus will not fertilize the eggs of the latter. The same property is present in the blood of the annelid and the mollusc.

If the two kinds of sperm are added simultaneously to the sea-urchin's egg all the eggs are fertilized. If the two sperms are mixed and kept together for two minutes, 60 per cent of the eggs are fertilized but do not form membranes; if the mixed sperm are kept together for four, six, or eight minutes the eggs are not fertilized. When sea water was added (after twenty minutes) to the last three sets, the eggs of the first lot formed membranes, but died at the beginning of the cleavage; the second lot was fertilized without membrane formation but died; the last lot gave no reaction.

If the mixture of the two sperms is kept from fifteen to twenty minutes, then added to the eggs and after five minutes fresh sperm of Sphaerechinus is added, membrane formation takes place, showing that the eggs had retained their power of fertilization for five minutes in the mixture. If the eggs were kept for thirty minutes in the mixture they could no longer be fertilized by fresh sperm.

The relative amount of Chaetopterus sperm that will antagonize the action of the sea-urchin's sperm, was tested by mixing them in different proportions. One part of Chaetopterus sperm to nine parts of sea-urchin sperm did not prevent fertilization, but two volumes of the former to eight of the latter and all further mixtures in this direction prevented fertilization. That the result is due to the "absolute concentration" is shown by diluting these mixtures with sea water when membrane formation takes place.

In order to test whether the results are chemical in nature and not dependent on the presence of living sperm, the Dentalium sperm was heated to ninety degrees, cooled, and mixed with Sphaerechinus sperm. When this mixture was added to the eggs of the latter a large percentage of the eggs lifted their membranes, but from ten to thirty per cent were not fertilized. To this extent the results were different from those when two unheated sperms were mixed.

That the sperm mixture in itself does not interfere with normal development after the eggs have once been fertilized, was shown by first fertilizing the eggs in sea water and then after five minutes putting them into the mixture where they developed normally. That this result was not due to the protection afforded by the fertilization membrane, that forms immediately on fertilization, was shown by shaking off the membrane as soon as formed, and then placing such treated eggs in the mixture. They developed normally.

The relations here shown do not hold for all other species. For example, the sperm of Asterias, holothurians and Antedon gave negative results, i. e., eggs in such mixtures were fertilized by their own sperm.

Godlewski compares the antagonistic action of the sperm of different species with analogous phenomena in the field of serology relating to the antagonistic action of foreign sera, for instance, with the work of Bordet and Erlich. According to the latter's side-chain hypothesis of haemolysis, the amboceptor unites by one side-chain with the receptor of the blood corpuscles and by the other side-chain with the complement. In this way the complement acts on the blood corpuscles through the medium of the amboceptor.

Herlant ('12) confirmed Godlewski's account of the antagonistic action of the sperms on each other, and extended the observations to other combinations. He suggested that the effects are brought about by changes in the surface of the egg. Loeb ('14) made a similar suggestion. But Godlewski has disproven this view by showing that if fresh sperm is added to the mixture in which the eggs have remained unfertilized, they will at once be fertilized. Lillie has proposed a different solution. He suggests that both the blood and sperm of a foreign species can bind the spermatophile receptor of the sperm of another species, hence

the sperm can no longer combine with its own fertilizin, which is necessary in order that it may fertilize its own egg.

The more recent results of Godlewski ('26) offer a simpler explanation of the antagonistic action of two kinds of sperm, namely: that their loss of power to fertilize is the result of mutual agglutination in the same sense that this term is used in serology and bacteriology. He shows that a certain length of time is required to complete the process lasting from twenty minutes to several hours, according to the combinations. The first indication of inactivation is shown by a decrease in the number of eggs that are fertilized by the mixture. The number fertilized gradually decreases until at last no eggs at all are fertilized. The result is interpreted to mean that only gradually are the sperm agglutinated. As long as some remain free the egg may be fertilized; the number fertilized being a measure of the sperm still free. The eggs in the mixture do not lose their power to be fertilized, for, if fresh sperm is added, they are immediately fertilized. Since the same results are produced if the sperm is heated to 100 degrees C., the effect must be due, not to the sperm, but to the fluid surrounding them. Foreign blood produces the same effect, and fluid from sperm that has passed through a filter will also inhibit the action of other sperm. The inactivation of one sperm by another is increased at a temperature of 30 degrees.

The loss of power of the sperm to fertilize eggs is, as has been said, interpreted by Godlewski to result from true agglutination, and is a phenomenon analogous to the agglutination of microbes and of the red corpuscles of the blood of one species by the blood of a different species.

CHAPTER V

SELECTIVE FERTILIZATION AND SELF-STERILITY

Whether the first spermatozoön that reaches the surface of the egg is received, or whether the egg is more receptive to one kind of spermatozoön than to another is not known from direct observation which offers many practical difficulties to investigations of this kind. One attempt of the sort on a very small scale has been made (Morgan, Payne, and Browne '10), but no evidence of selection was observed. A few eggs of Cumingia were mounted in a drop of water and a few sperm introduced at one side of the cover-slip. The first sperm, that bored through the jelly to the surface of the egg, entered it. This result needs confirmation on a larger scale, for there are obvious sources of error such as sperm entering in oblique paths, or above or below the field of actual observation, etc.

On the other hand, genetics furnishes very convincing evidence that in all ordinary situations the egg does not discriminate in its receptivity between spermatozoa that carry different heredity factors. The whole modern theory of Mendelism rests on the assumption that no "choice" is shown, and the results of genetic studies fully bear out this point of view. It is not, of course, to be expected that such differences as those characterizing most Mendelian genes should call forth differences in the fertilizing ability of the sperm. Whether there may not be other kinds of difference in the spermatozoa that make them individually more or less likely to reach the egg before other spermatozoa of the same individual do so, is another question. For example, it is conceivable that the female-producing sperm containing an X-chromosome, are slower in their passage up the tube of the oviduct of the bird or mammal than are the male-producing sperm with the smaller Y-chromosome, or with no sex-chromosome at all. If this should occur, more eggs might be fertilized by the latter, because these sperm would be more numerous in the upper

reaches of the oviduct. It might be possible in this way to explain the sex ratio in man where to each one hundred female births there are about one hundred and six male births on an average. But it has not been shown that such differences in rate of motion occur, and there may be other explanations of this sex ratio.

In the breeding of certain races of the pomace fly, Drosophila, melanogaster, there are a few cases that appear at first sight to indicate that selective fertilization occurs (Morgan '12), but closer analysis (Lynch '19) has shown that a different interpretation is more probable. For example, in the sex-linked mutant type called fused, no offspring are produced when fused males are mated to fused females; but fused females will produce female offspring if bred to wild-type males, or to males of other mutant types. Moreover, if a female (F_1) heterozygous for fused is bred to a fused male, the offspring are of four kinds, viz., heterozygous fused females (wild type in appearance), fused females, wild type males, and fused males, in the expected proportion of 1 : 1 :: 1 : 1. This result makes it clear that the fused-bearing sperm can unite *under these conditions* with fused-bearing eggs to produce pure, fused females and males. Why then does not the same combination form when fused is bred directly to fused? The difference in the two cases must obviously be in the heterozygous nature of the eggs before maturation when a heterozygous female is the mother. When the polar bodies are formed in such eggs either X-chromosome may be thrown out into the polar body, consequently two kinds of "reduced" eggs are produced, one with the normal (i.e., the wild-type X) and the other with the fused-bearing, X-chromosome. Both eggs have been in the ovary under the influence of a normal X. Each of these, if entered by a fused sperm, develops; one to produce a heterozygous female and the other a fused female. Similarly when these two kinds of eggs are fertilized by the Y-sperm normal males or fused males are produced. In other words, an ovarian egg that has developed in the presence of the normal allelomorph for fused, even if that gene is extruded in the polar body, will develop if a fused sperm enters, but an ovarian egg that has developed in the presence of two genes for fused does not develop if fertilized by a fused-bearing sperm. Hence it is not selective fertilization but a different phenomenon that explains the results, provided it could be shown that the fused sperm enters the egg in the case when fused is

mated to fused. Miss Lynch found, in fact, embryos present in the egg-shell in cases when fused was mated to fused. But after the second day she found that the eggs began to blacken and then died. Only two larvae "out of hundreds" hatched, and those two died before pupation. It is probable, therefore, that the fused sperm enters the fused egg, but if the ovarian egg has developed under the influence of the fused genes only, development does not take place further than the earlier stages. The phenomenon appears to rest on a protoplasmic basis, because, when an ovarian egg has developed in the presence of a normal and a fused gene, such an egg develops into a fly when fertilized by a fused-bearing sperm, although it has lost its normal gene in the polar body. The influence of the normal gene remained. The experiments also show why females only are produced when pure fused females are outbred to wild-type males. The latter bring in a normal X-chromosome that restores the protoplasm through its activity sufficiently to overcome the earlier deleterious influence of the presence in the ovarian eggs of two fused genes. Males do not develop because the Y-bearing sperm brings in only a Y-chromosome which does not carry genes normal to fused.

An apparently parallel case is found in the genetics of yellow mice, but the situation is somewhat different because the pure homozygous, yellow type does not exist and there is therefore no opportunity to study the "prematuration influence" which is the essential point in the case of fused. The facts for yellow mice are as follows: A yellow female mouse mated to a yellow male gives yellow mice and mice of another color in the ratio of two yellows to one not-yellow. The yellow offspring give the same result. Moreover, yellow by not-yellow gives half yellow, half not-yellow offspring. Evidently the yellow mice are always heterozygous (YC), no pure yellows (YY) exist. The gametes of the yellow mouse (YC) undergo ordinary Mendelian segregation (as shown by the back-cross that gives equal numbers of yellow and colored offspring). It was at first supposed that the results could be explained by selective fertilization, that is a yellow-bearing sperm could not fertilize a yellow-bearing egg, but other sperm could do so. The expectation would then be, for yellow by yellow, three yellows to one not-yellow. Cuénot first showed that the actual ratio is nearer 2:1, and later results by Castle and Little have shown that the ratio is actually 2:1.

The ratio is compatible with the expectation that the yellow-bearing egg is entered by a yellow-bearing sperm, thus preventing any other sperm from coming in. These embryos die. Thus only two terms of the Mendelian F_2 ratio (1:2:1) remain, viz., the last two. All yellows that survive belong to the heterozygotic class, while the non-yellow does not carry yellow. Moreover, Little ('17) has shown more recently that a certain number of embryos begin to develop and then die and are absorbed *in utero* when yellows are bred to yellows, and that such embryos are more frequently found than when yellows are bred to non-yellows. These abortive embryos are presumably the pure yellow (YY) combination. It is quite clear, therefore, that selective fertilization does not take place.[1]

In flowering plants the pollen tube with its two (or three) contained nuclei "grows" down through the tissues of the style to reach the ovules. The length of the style traversed is often very great as compared with the size of the pollen grain. It is generally believed that the so-called growth is made possible by some chemical reaction taking place between the end of the pollen tube and the tissues of the style.[2] Any change in the composition of the one or of the other may be expected to affect the rate of "growth," and in consequence increase or decrease the chance that a pollen grain of given constitution may reach the ovules. Hence the possibility of a different rate of fertilizing power of pollen grains having different genetic compositions. The pollen grain is haploid and comes from a "reduced" cell while the tissue of the style is diploid.

Correns ('18, '21, '27) has shown in the dioecious plant, Melandrium (which has more recently been shown to produce

[1] In some snails there are two kinds of spermatozoa formed, typical and atypical; and in some of these cases it is known that many eggs fail to develop and become food for the snails that do develop in each capsule. Hyman ('26) obtained evidence in the snail Fasciolaria, that the atypical sperm fertilize the eggs, in the sense that they cause the fertilization membrane to be thrown off which prevents the entrance of the typical sperm. As many as 97 per cent of the eggs of this snail fail to develop. Portmann ('26) has found in Buccinum that the atypical sperm enter the egg and form a sphere that may contain one or more asters. The sphere moves into the vicinity of the polar spindle whose disintegration then follows, thus preventing normal development.

[2] The failure to self-fertilize, that is a special case of selective fertilization, will be treated in the next section.

dimorphic pollen) that the sex-ratio is different when many or a few pollen grains are placed on the stigma. When many grains are present there are more female plants produced; when fewer are present there is a nearer approach to equality of the sexes. The difference can be explained on the assumption of the relative rates of growth of the two kinds of pollen. When many pollen grains are present, a higher percentage of the eggs would be fertilized by the female-producing grains if these grow a little faster than the male-producing grains. But when only a few grains are present there may be not enough female-producing grains to fertilize all the eggs, hence the more slowly growing grains have a better chance of finding eggs, that are still unfertilized, when they reach the ovary.

Self- and Cross-Fertilization

The term self-sterility can, of course, be appropriately applied only in those cases where eggs and sperm are produced by the same individual. The most familiar examples of self-sterility are found amongst those flowering plants having stamens and pistils on the same plant. Some of these are known to be self-sterile; others, however, may be self-fertile. Several groups of animals are hermaphroditic, but self-fertilization has been tested in relatively few of them. In those species of nematodes that produce both eggs and sperm in the same individual, self-fertilization is known to take place. In the cestodes self-fertilization probably occurs. The land snail, Helix nemoralis, has been shown by Lang to be self-fertile, and the air-breathing pond snails (Lymnaea, Physa, Planorbis) have also been shown by Colton ('12, '18) to be capable of self-fertilization.

The ascidians are hermaphroditic. In several species (Molgula, Cynthia) self-fertilization is known to take place, but in another species, Ciona intestinalis, self-sterility occurs.

Castle ('96) observed in the American strain of Ciona intestinalis, that the eggs are, as a rule, incapable of fertilization by sperm of the same individual, but he also recorded instances in which 90, 25, 10, 5 and 4 per cent of the eggs were self-fertilized. My own observations ('04, '10) have not confirmed the higher numbers, but have shown that, at best, only a very small percentage of eggs are self-fertilized; as a rule, none are fertilized.

Castle also showed that eggs of one individual may be fertilized by sperm from any other, but it is possible that there are various degrees in this cross-fertility. Fuchs and Potts reported that the same species of Ciona found at Naples is self-fertile in a high degree.

In Ciona, as in other ascidians, there is a sac-like ovary to the wall of which the eggs are attached. As the eggs ripen they fall into the interior of the sac and then pass out into the long oviduct where they accumulate. The testis consists of a number of follicles in the vicinity of the ovary that open into tubules converging to the single vas deferens which takes a parallel course to the oviduct, opening near its terminus. Eggs and sperm are extruded in the early morning. Normally fertilization takes place in the sea, and, to judge from experimental results, the eggs are fertilized by sperm of other individuals set free at the same time.

The mature egg is covered with a layer of tall pointed cells, called follicle cells, each with a clear drop (oil) near its end. These cells rest on a thick membrane, probably perforated by small pores. When set free the egg fills the interior of the membrane, but it soon shrinks away from the wall, and then, many cells can be seen lying singly or in groups outside the egg, but inside the membrane. These are the test-cells that arise at an early stage in the egg's history from the follicle around each egg. A spindle, with chromosomes in the metaphase, is present in all eggs in the oviduct. The egg remains in this condition until it is fertilized, when the first and then the second polar body is extruded.

If the eggs are taken from the oviduct and mixed with sperm from the vas deferens of the same individual, none of the eggs are, as a rule, fertilized, but if the sperm from another individual is added, the eggs are immediately fertilized, and begin to segment in less than an hour. If the eggs have stood longer the segmentation begins sooner.

The failure of sperm to fertilize the eggs of the same individual, although it can fertilize eggs of most other individuals, is the peculiar feature of the situation. In plants this phenomenon is well known. It has been shown that the pollen tube does not grow with sufficient rapidity in its passage through the tissue of its "own" pistil to reach the ovaries. It has also been shown that the pollen will fertilize its own eggs should it reach them,

as may happen under special circumstances. For instance, East discovered that self-fertilization may take place in self-sterile tobacco plants at the end of the breeding season, which shows that union between the egg and its own sperm is possible at least at this time. Also if ripe pollen is placed on the stigma of young flowers (before they open) of the same plant it may sometimes succeed in reaching the eggs and in fertilizing them. In Ciona the sperm has only to pass between the follicle cells and through the membrane in order to reach the space around the egg in which the test-cells lie. Sections of self-fertilized eggs (Morgan '04) show that the sperm actually gets into this fluid space, but does not enter the egg. Thus, failure to bring about segmentation is shown not to be due to inability to pass through the membrane, but to failure to unite with the egg after passing into the region around the egg.

The many different kinds of experiments that were made by Morgan to detect the cause of the failure of the sperm to fertilize "its own eggs" need not be described, since most of them led only to negative results. It was later discovered (Morgan '23) that the whole egg, or a fragment of an egg, could be self-fertilized if the egg is first freed from its membrane (and the surrounding cells and fluid). This proved that there is something in or produced by the enveloping cells that prevents self-fertilization. For example, if the membranes are torn open by means of small sharp needles, the egg may be squeezed out into the water. Sometimes the whole egg comes out at once, often only a part. The fragment may be large or small. It rounds up into a sphere. If sperm from the same individual is now added, practically all of the extruded eggs, and even the fragments, become fertilized and segment into two, then into four, eight cells, etc.

It is improbable that the outer follicle cells are responsible for the inhibition since they may be shaken off the eggs without bringing about self-fertilization. Whether the spermatozoa are brought to rest by the perivitelline fluid, or whether their activity is so altered by this fluid that they fail to penetrate cannot be stated. There is some evidence, though quite insufficient, showing that certain substances such as alcohol or ether, that excite the sperm to greater activity may cause, at times, a higher per cent of self-fertilization to occur. It may seem not improbable, therefore, that the action of the perivitelline fluid is to make the sperm

quiescent, but that this action may be overcome if the sperm are exceptionally active.

In his earlier work Morgan ('04) obtained evidence showing that while the sperm of one individual may fertilize all the eggs of other individuals, yet in many cases only some of the eggs are cross-fertilized.

Ciona intestinalis found at Naples is not self-sterile to the same extent as is the American strain or variety. Potts ('13) has recorded that if the eggs are heavily inseminated with sperm of the same individual, nearly 100 per cent of the eggs develop. Fuchs ('14) has obtained similar results although it was "comparatively rare" in his experiments that all the eggs were self-fertilized.

Fuchs has carried out many experiments in self- and cross-fertilization with the Neapolitan form. He lays much emphasis on the concentration of the sperm-suspension, finding that the more concentrated the suspension the greater the number of eggs self-fertilized. He suggests that it is due to neglect of this precaution that many of the results described by Morgan may have arisen. It may be pointed out, however, that this possibility was fully appreciated by Morgan, who states that as far as posssible equivalent amounts of sperm-suspension were used whenever comparisons were to be made. It may be further stated that in the American form there are no such differences in the number of eggs self-fertilized as are recorded by Fuchs for the Neapolitan form, no matter how concentrated the sperm-suspension may be. Fuchs found in an experiment with 4 individuals that 5 drops of a suspension gave 0, 0, 2, 0 per cent self-fertilization, but with 4 cc. of the same suspension 58, 22, 100, 56 per cent of the eggs were self-fertilized. In another similar experiment 2 drops gave 2 per cent; 25 drops 24 per cent; and 10 cc. of the suspension gave 100 per cent. In another experiment 1 drop gave 0 per cent; 5 drops 1 per cent; 20 drops 3 per cent; 100 drops 11 per cent. In a fourth experiment 3 drops gave 1 per cent; 1 cc. gave 1 per cent; 5 cc. gave 47 per cent; and 10 cc. gave 89 per cent. Despite the variability here shown it is obvious that the greater the number of spermatozoa, the greater the chance of self-fertilization. The eggs of these same individuals were fertilized with the same amounts of solution from another individual and in nearly every

case 100 per cent of the eggs were fertilized. Evidently cross-fertilization is much more likely to occur in the Neapolitan form than self-fertilization, even although self-fertilization gives a fairly high degree of fertilized eggs. Fuchs confirmed Morgan's observation that eggs segment sooner if they have been kept for some time in sea water before they are fertilized. He found further that if the later fertilization was not too prolonged, the embryos hatched sooner than do those from eggs fertilized at once. Eggs from different parts of the oviduct did not show any appreciable difference in time of segmentation. The same relation held for the sperm. Self- and cross-fertilized eggs segmented at the same time. Cross-fertilized eggs produced embryos that settled down and lived longer (20 days) than did self-fertilized eggs.

Morgan ('04, '05, '10) made many experiments to test whether the eggs of a given individual are cross-fertilized to the same extent by sperm from other individuals. The amount of sperm used in each set was far in excess of the amount that would have fertilized all the eggs in the most successful cases. Fuchs, as stated above, has raised the objection, that, since in the Neapolitan form, the amount of sperm suspension in self-fertilization is an important element in determining the amount of self-fertilization, unless Morgan's results were carefully controlled for this factor his results are invalidated. The objection is well taken and bears on the problem in question; for, if it were true that equivalent amounts of sperm had not been used, it is possible that different numbers of eggs would have been cross-fertilized. But this objection had been foreseen and pains taken to obviate it. Furthermore, a later experiment ('23) made to test how far the sperm of the Woods Hole Ciona must be diluted to affect the number of eggs cross-fertilized has shown that the dilution is far below anything used in the earlier work.

The following tables may serve as a sample of the kind of results obtained by cross-fertilization in the American type of Ciona. The eggs of an individual (A) were taken from the oviduct, divided into five nearly equivalent lots and placed each in a given quantity of sea water. Similarly for B, C, D, E. The sperm was then taken from the first individual, mixed thoroughly in sea water and the same number of drops added to each of the kinds of eggs (A, B, C, D, E). The small letters above the

capital letters in the table indicate the sperm added to each. In each table there is a diagonal line (A^a, B^b, C^c, D^d, E^e) of self-fertilized lots in which no eggs segmented.

TABLE VII

A^a 0	A^b 10	A^c 40	A^d 50	A^e 0	A^f 40
B^a 30	B^b 0	B^c 90	B^d 80	B^e 1	B^f 25
C^a 90	C^b 95	C^c 0	C^d 99	C^e 100	C^f 100
D^a 90	D^b 90	D^c 80	D^d 0	D^e 1	D^f 99
E^a 0	E^b 40	E^c 25	E^d 50	E^e 0	E^f 0
F^a 1	F^b 40	F^c 80	F^d 15	F^e 0	F^f 0

In Table VII the C-eggs gave almost 100 per cent fertilizations as did the D-eggs also, except D^e that gave only 1 per cent. That the e-sperm was good is shown by C^e. Neither the A nor the E eggs gave, at best, more than 50 per cent fertilized eggs, while the B and F eggs gave both high and low percentages. The results are probably due in part to the poor condition of the eggs in the A and E individuals, but the differences in the other lots are possibly significant.

TABLE VIII

A^a 0	A^b 87	A^c 92	A^d 84	A^e 96
B^a 38	B^b 0	B^c 35	B^d 98	B^e 97
C^a 93	C^b 96	C^c 0	C^d 97	C^e 96
D^a 91	D^b 98	D^c 77	D^d 0	D^e 89
E^a 96	E^b 92	E^c 60	E^d 74	E^e 0

In Table VIII another series is shown. Some of the combinations in each set show a high per cent of fertilizations approaching 100 per cent, but there are several cases in which a much lower percentage of fertilizations occurred. A more extensive and better controlled experiment will be necessary in order to prove that these differences are really due, as appears to be the case, to certain combinations being formed more readily than are others, or whether due to accidental conditions such as the immaturity or over-ripeness of the eggs, etc.

Before the discovery ('23) that eggs freed from their membranes could be self-fertilized a large number of experiments had

been made by Morgan to find out if possible the nature of the block. Although, as stated, most of the experiments gave negative results, a few at least are significant in the light of the later work. The eggs of an individual (A) were removed and soaked in an extract of the ovary of another individual (B). Then (A) sperm was added. No fertilization occurred. The reciprocal experiment gave the same result. Evidently there is not produced anything in the ovary of a different individual that makes self-fertilization possible, or if so it does not affect the eggs of other individuals under the stated conditions. Similarly eggs allowed to stand in the blood of another individual, and then inseminated with their "own" sperm, were not fertilized.

Again, the eggs of (A) were mixed with an equal number of eggs of (B), then sperm of B was added. Half the eggs segmented (presumably the A eggs). It follows that the eggs of another individual do not set free any substance that will remove the block against self-fertilization. In another experiment A-eggs were soaked in extract of B-ovary and later B-sperm added. Fertilization occurred, showing that the extract in itself had not interfered with cross-fertilization. If, however, the extract is too concentrated even cross-fertilization is interfered with, and this is also known to hold in unisexual forms. Strong concentration of any body fluid, by stopping the activity of the spermatozoa or possibly by locking up the sperm, interferes with normal fertilization. In the case of Ciona the concentrated fluid does not injure the sperm; for if the concentrated extract is afterwards diluted with sea water fertilization takes place.

Two results were obtained by Morgan in 1905 that foreshadowed the later result in which eggs freed from their test and follicle cells were self-fertilized. By shaking the eggs violently in a small tube, so that many of the follicle cells were removed (and perhaps the membrane broken), or some of the perivitelline fluid diluted, some eggs were self-fertilized—as many as 20 per cent in one case—although this did not always happen. Again by compressing the eggs between a cover-slip and slide the egg membranes were broken in some eggs. Many of the eggs that had been broken were self-fertilized "indicating that the resistance to self-fertilization is due to something in the membranes surrounding the eggs." A few years later Dr. Kite, at my request, tore open the membrane of three or four eggs by a microdissection needle,

and then brought some of their "own" sperm into the opening. All the eggs so treated were self-fertilized. These results, taken in connection with the later ones ('23) described above, leave no doubt that the block to self-fertilization does not lie in the egg itself, but in the fluids or cells around the egg.

A series of experiments (Morgan '10) on the effect of cold on self-fertilization were made in order to determine whether the lower temperature would affect the results. The eggs of Ciona were subjected to a temperature of 0° C., or even lower, then returned to room temperature, and while still cold sperm was added. In one case 50 per cent of the eggs were self-fertilized, after the eggs had been on ice for one hour, but none were fertilized in one such set that had become warmer. In another case after two hours' exposure to cold, 98 per cent of the eggs were self-fertilized. When salt was mixed with ice to give a still lower temperature one set, after two hours in the cold, gave 50 per cent, but another, after four and a half hours, gave only 1 per cent, while four other sets gave nothing. These results were so irregular that unless repeated, with controls, it is doubtful if much value can be ascribed to the apparently successful cases.

In order to find out whether prolonged washing might remove inhibiting substances produced by the egg, or by its membranes, the following experiment was made. Eggs were placed in a jar through which air was bubbled, keeping the eggs in circulation. After three hours the eggs were removed and their own sperm added. Many of the eggs divided, but as a rule into unequal parts, and the cleavage went no farther. The results are due to artificial parthenogenesis,[3] as other experiments described below made evident.

In order to test the possibility that self-sterility might be in the nature of immunity, the following experiments were made (Morgan '10). The terminal portion of the oviduct (sometimes with the sperm duct also) was ligated at two levels and the piece cut out. It was inserted into the body of another individual between the test and body wall and left there for several hours (5, 19, 24 hours), then removed. The eggs were taken out and self-fertilization tried. In many cases the eggs segmented, but generally only once and into unequal parts. Cleavage then

[3] It is possible that the two cells correspond to the first polar division, and, if so, the smaller cell may be the first polar body.

stopped. If the eggs were not left too long in the host they could be cross-fertilized. The sperm also was shown to have not lost its power to cross-fertilize the eggs of the host.

The cleavage that took place after "grafting" was obviously in the nature of parthenogenetic development, incited by the blood of another individual. No control was made, however, to test whether if grafted into the same individual parthenogenesis would occur. The action of foreign blood in inciting to artificial parthenogenesis has been described by Loeb. It is, however, worthy of note that this occurred in Ciona, since, as Lyon ('03) has shown, the ordinary methods of bringing about parthenogenesis do not work well with ascidian eggs.

The eggs of another ascidian, Cynthia partita, are more often self-fertilized, but still more eggs segment if sperm from another individual are afterwards added. In these respects this species behaves in much the same way as the Naples variety of Ciona. Individual Cynthias were isolated. Those that emitted sperm and eggs gave the following percentages of self-fertilizations: 33, 10, 100, 95, 95, 75, 10, 30, 30, 10, 1, 75, 40, 85, 33, 0, 10, 4, 4, 0, 12, 4 per cent. Without further evidence these results might be ascribed to insufficient sperm but this seems very improbable. When cross- and self-fertilization were made, more eggs were fertilized in the crossed sets than in those self-fertilized—in fact self-fertilization often failed. Owing to the irregularities in the results obtained it may be well not to lay much emphasis on these differences.

In another ascidian, Molgula, self-fertilization is as easy as cross-fertilization. It is evident from this that self-fertilization is not a result of hermaphroditism itself, but must be due to a special relation that has been superimposed on these hermaphroditic forms. This holds for plants also.

Fuchs ('14) has carried out experiments, many of them like those described above, on the Naples variety. He found that extracts of the eggs obtained by crushing caused more eggs to be self-fertilized in extracts of a given concentration than in the control in sea water. But the same results occurred whether the extract was from its "own" ovary or from that of another individual. The results indicate that the increase in number of eggs fertilized is here not a specific reaction but due perhaps to the extract increasing the activity of the spermatozoa so that

more succeeded in passing the block. Fuchs found that extract of the eggs of another ascidian, Phallusia, as well as of two species of sea-urchins gave the same result.

Water in which eggs have stood for some time (egg-water) also has a similar activating effect on the sperm of Ciona. An addition of 1 cc. 1/10 NaOH to 200 cc. sea water increased the percentage of cross-fertilizations, while the addition of a small amount of acid (HCl) decreased the percentage.

Conclusions

In recent years many experiments have been made with plants, particularly by Correns ('12), East ('15-'25) and Compton ('12, '13), in which it has been shown that the breeding results are compatible with the assumption that factors for self-sterility occur. Correns ('12) first experimented with the self-sterile plant Cardamine pretense. He crossed two plants B and G, and reared their offspring. He then back-crossed the offspring to each of the two parents, and found in each case that about half the back-crosses were fertile, half sterile. The result he explained as follows: If a factor is assumed to be present in B that prevents self-fertilization, this same factor B, being present in half of the F_1 offspring, half of them should be cross-sterile with the B parent. Similarly for the other factor G, half of the F_1 offspring should be cross-sterile with G. The explanation is incomplete in so far as one of the expected F_2 types (b, g) should be self-fertile which was not demonstrated.

East ('15) made an extensive series of experiments with self-sterile tobacco and advocates a view similar to one suggested by Morgan for Ciona. By suitable breeding tests he has shown that cross-sterile plants are produced in later generations, when two species, each self-sterile, are bred together. Thus in the F_2-generation, individuals are expected that are inter-sterile, and such were found. On the assumption that some of the factor-differences between the species may be linked, East found that the actual results were in harmony with expectations. Crossing over would give new combinations not present in the two original types, each assumed to be homozygous for the postulated allelomorphic genes.

Later East and Park ('17, '18), and East and Mangelsdorf

('25), have further analyzed the problem of self- and cross-fertility. In order to carry out the experiment more accurately, hybrid offspring from two species of self-sterile plants, Nicotiana alata and N. forgetiana, were made homozygous by inbreeding through twelve generations. Several such families were obtained that were homozygous or approximately so. The conditions in one of the resulting families were as follows. Three kinds of individuals *a*, *b*, and *c* were found. Each individual of any one of these kinds is sterile with any other individual of the same class and fertile with each individual of the other two kinds; but the progeny resulting from reciprocal crosses is different. Thus, *a* ♀ by *c* ♂ gives *b* and *c* individuals only, while *c* ♀ by *a* ♂ gives only *a* and *b* individuals. Two classes always appear in equal numbers, but the class of the mother is never represented in the offspring. The explanation is as follows. If three allelomorphic genes, S_1, S_2, S_3, are present in such a family and if class $a = S_1\ S_3$; class $b = S_1\ S_2$; class $c = S_2\ S_3$ and if the pistil of the plant affords stimulus for the growth of the pollen which bears sterility factors other than its own, the results find a consistent explanation. For instance, plant *c* ($S_2\ S_3$) affords a sufficient stimulus only to pollen carrying factors other than $S_2\ S_3$. Only pollen bearing the factor S_1 can penetrate the style and fertilize the eggs. The progeny will be $S_1\ S_2$ (class *b*) and $S_1\ S_3$ (class *a*) in equal numbers. Reciprocally, *a* ♀ ($S_1\ S_3$) by *c* ♂ ($S_2\ S_3$) permits the S_2 pollen alone to penetrate the eggs giving $S_1\ S_2$ (*b*) and $S_2\ S_3$ (*c*). This result, which is typical of all the others, explains why the maternal combination is absent in the progeny; why the progeny of reciprocal crosses is different; and why the progeny counts of two classes other than the maternal are equal regardless of which of the other two classes serves as the male parent.

There are several ways of testing the validity of this hypothesis. The tests have been made and the hypothesis confirmed. This convincing analysis, the result of carefully planned genetic experiments, is a contribution of the first rank to a problem that has baffled students of fertilization for seventy-five years and more. The solution is not only a keen genetic analysis of the case, but gives an insight into the physiological reaction between the haploid pollen tube and the diploid tissue of the female. It has been shown by direct observation that the rate of growth of

the pollen tube in the tissue of the female is consistent with the view that a differential rate of growth is actually present. The nature of the reaction is not known at present, but may reasonably be assumed to be chemical.

Compton ('12, '13) found both self-sterile and self-fertile plants of mignonette. Certain self-fertile plants when self-fertilized threw three self-fertile to one self-sterile offspring. If the same plant were crossed to a self-sterile plant, self-fertile and self-sterile plants were produced in the ratio of one to one, etc. The results are explicable if there is a single factor difference, a dominant factor for self-fertility and its allelomorphic factor for self-sterility.

Morgan ('04) had suggested an interpretation for Ciona based on genetic possibilities. Briefly it was this. Individual Cionas differ from each other in certain genes having to do with self-sterility. In any given individual the genes are the same in every cell, including the germ cells, before maturation. An individual may be heterozygous for some or for all of these genes but the protoplasm of every cell including egg and sperm cells (before maturation) has been under the influence of the same group of factors carried by that particular individual. The statement that each individual of Ciona is self-sterile means in a physiological sense that when the eggs and sperm have developed under the same genetic complex, (not necessarily however homozygous in its factors), self-fertilization does not take place. It follows that all individuals having the same genetic complex should not only be self-sterile but should be also cross-sterile. We should expect, then, to come across at times two or more such individuals. How frequently they would occur would depend on how many factor differences are present in a given race. If only a few such differences are present many such cross-sterile individuals are expected; if many such differences are present, relatively few such individuals are expected. In Ciona we do not know how often this happens.

So far the discussion is based on the view that any two individuals identical in their genes (whether heterozygous or homozygous for a given complex) are cross-sterile for the same physiological cause that makes them self-sterile. There is, however, in the case of Ciona the more difficult question as to whether amongst the different kinds of individuals there may be degrees

in which they are cross-fertile. This is the condition that my results seem to indicate. The facts are admittedly not sufficient to give the essential data for such a complex theoretical situation, yet there are certain possibilities that may be referred to, that make such a relation not incompatible at least with the hypothesis suggested above.

It is possible, for instance, to suppose that this is a multiple factor case. Then individuals that differ in several or many factors might be cross-fertile and other individuals differing in fewer factors might be so nearly alike that cross-fertility would be less likely to occur. Such a view could only be established by isolating such types and testing them by suitable crossings. The situation has been found too difficult to handle at present with this material; for, it would involve raising several generations to maturity.

The further question as to whether the amount of sperm would also play a rôle in such a situation, assuming sufficient to be always present to insure full fertility when the maximum genetic differences were present, opens up further problems even more difficult to consider in the absence of adequate data.

CHAPTER VI

PARTIAL FERTILIZATION

The term "partial fertilization" was applied by Boveri ('88) to cases in which the sperm-head, after it has entered the egg, does not move fast enough through the cytoplasm of the egg to reach the egg-nucleus at the time the latter divides. The sperm-aster, however, has in the meantime reached the egg-nucleus, and has given rise to the segmentation spindle (Figs. 251–253). The first division of the egg-nucleus and of the egg may take place while the sperm-nucleus remains detached and condensed. The failure of the sperm-nucleus to pass to the center of the egg may be due to its failure to absorb fluid and enlarge. It is carried passively into one of the blastomeres when the egg divides, and may later reach the daughter nucleus of the blastomere that contains it, and at the next division combine with the daughter nucleus and take part in the division of the blastomere that follows. As a result, one of the blastomeres divides with a diploid number of chromosomes (a maternal and a paternal set), while the other blastomere divides with only a haploid (maternal) set of chromosomes. Boveri also found that the sperm-nucleus may not become incorporated into a dividing nucleus until the 4- or 8-cell stage. Teichman ('03) has made similar observations. Furtheremore, the same phenomena have been observed in cases where the egg is started to develop by parthenogenetic agents and later fertilized. These cases will be discussed in connection with parthenogenesis.

Partial fertilization has not only several interesting embryological implications, but has been found important in explaining cases in genetics where mosaics that are haploid in one region, diploid in another, have been reported.

Whether the following cases are or are not to be included under the term partial fertilization is, perhaps, only a question of definition, but since they also show an influence that the entrance

of the sperm has induced in the cytoplasm of the egg without participating in the result, they may conveniently be treated here. Boveri ('95) shook eggs of the sea-urchin to pieces a few minutes *after* the sperm had entered. Some of the fragments were found to contain only the sperm-nucleus and its accessories. These fragments underwent division, the sperm-aster giving rise to the mitotic figure on whose equator the sperm-chromosomes (haploid) became arranged and separated as in normal division. Cytoplasmic division followed.

The fragments that did not contain a sperm-nucleus, but did contain an egg-nucleus, did not divide, but the egg-nucleus enlarged and around it the cytoplasm formed a large monaster. The nuclear wall disappeared, and the chromosomes were set free and they divided, but, in the absence of a bipolar spindle, remained in situ. A new nuclear wall formed around the chromosomes, and a resting nucleus was formed. After a period of rest, the nucleus and the surrounding protoplasm may again show signs of activity at a time synchronous with the second division of the monospermic fragment. This may happen even a third time. Evidently the entrance of the sperm has affected the cytoplasm and egg-nucleus in such a way that they are started along the usual path of activity that would lead to the incorporation of the chromosomes on the spindle derived from the sperm. In the absence of the spindle, the egg-fragment is unable to form a bipolar spindle of its own, hence no division takes place. The result shows, in this egg at least, that the sperm arouses the egg-nucleus to activity as well as the cytoplasm, but the action fails to reach the division stage in the absence of the sperm-aster. The experiment shows one phase of the action of the sperm on the egg.

Similar results were obtained from an experiment of Ziegler's ('98). The egg, immediately after fertilization, was constricted by a cotton fibre (Fig. 5). One part contained the entering sperm, the other the egg-nucleus. A bridge of protoplasm connected the two portions of the egg. The bridge remained in some cases throughout the subsequent changes. In this instance the two portions remained in actual continuity, but the constricted region did not allow the sperm-nucleus to join the egg-nucleus. The results were, however, exactly the same as when the two parts were separated, as in Boveri's experiment. The portion containing the sperm-nucleus divided, that containing the egg-nucleus

did not divide, but the nucleus enlarged, its chromosomes were set free and a monaster developed. Here, even although there is actual continuity of protoplasmic substance, the egg-nucleus and its surrounding protoplasm fail to carry through the division, although their activity shows that the entrance of the sperm had initiated in them all the symptoms of division.

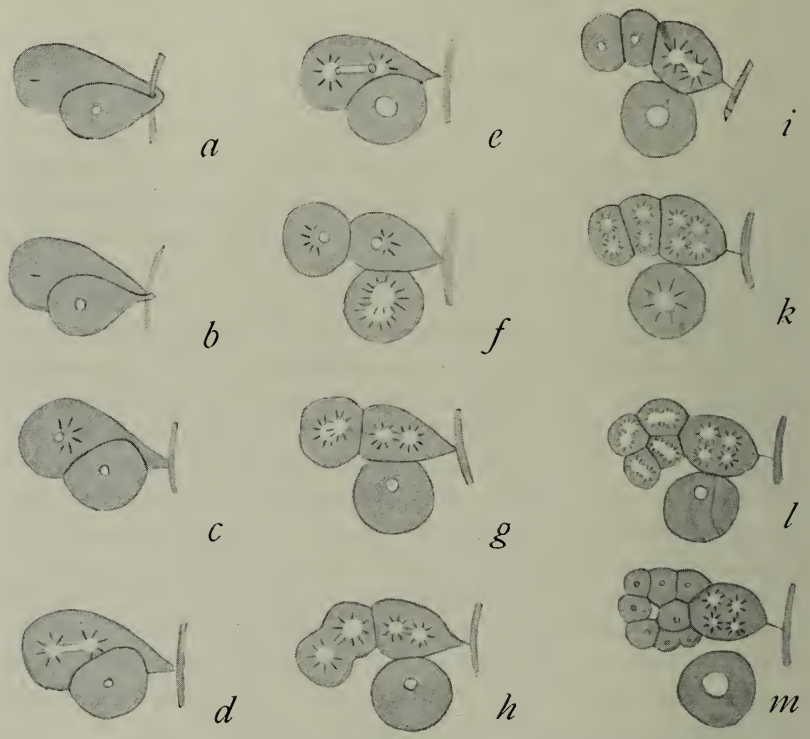


FIG. 5.—Constriction of the egg of the sea-urchin immediately after fertilization, by means of a cotton fibre, assisted by a stream of water. (After Ziegler.)

This experiment of Ziegler was made as follows: Pieces of raw cotton were placed on the glass stage of a compressorium. A drop of sea water with eggs was placed on the cotton. Another drop with sperm was placed on the cover-glass which was then lowered until the two drops united. The cover-glass was still further lowered until the distance between the two glasses was a little less than the diameter of the egg. Then a stream of water was turned on between the glasses (the compressorium was made so as to permit this). If the stream was of the right strength,

an egg might be drawn across a thread (Fig. 5*a-d*) and constricted into two parts. The current was then slackened, or if desired continued until the egg was actually divided into two pieces. By watching the eggs under the microscope during this process one or more might be found in which the sperm was in one portion and the egg-nucleus in the other. Ziegler studied both those cases in which the two pieces were pinched apart soon after fertilization, and those cases in which the two pieces remained connected by a neck until at least the first cleavage took place in the piece with the sperm. When the first cleavage took place the pieces separated from each other.

A fertilization membrane formed on both portions, and although not explicitly stated, it appears that, even had the constriction taken place before the spermatozoön entered, the membrane would develop on both portions, showing that this effect of the sperm could be carried through the connecting bridge. The influence, however, is not enough in itself to start development.

The effect of penetration of the surface layer of the egg of Nereis by a sperm without subsequent entrance of the sperm has been described by F. R. Lillie ('11). The result is somewhat complicated by the need of centrifuging the egg in order to remove the attached sperm; for, centrifuging itself introduces in this case somewhat comparable changes to those induced by the partial fertilization of the sperm.

A single sperm becomes attached to the fertilization membrane of the egg immediately after insemination, the jelly is poured out from the cortical layer, and a fertilization cone develops at the point of contact (Fig. 6*a*). The sperm takes about 54 minutes to enter the egg, during which time the fertilization cone gradually withdraws. If the eggs, with the sperm attached, are centrifuged from 30 to 50 minutes after they have been inseminated, a considerable number (20 to 95 per cent) fail to segment, while all the eggs of the control segment. A study of sections of these eggs shows that the centrifuging has carried off some of the sperm that were attached, along with the surrounding jelly (Fig. 6*b*, *c*, *d*). No sperm-nucleus is found in many of the centrifuged eggs, nevertheless they have given off both polar bodies, and the egg-pronucleus (without a spindle) is present in the eggs. Lillie concludes that the sperm induces some of these changes without itself entering the egg, but the effect goes only as far

as the formation of the egg-pronucleus. The absence of the sperm-aster, which is normally introduced with the sperm, may explain why the development did not proceed beyond this point. The chromosomes that have been left in the egg, usually form individual vesicles that do not unite perfectly and later become scattered in the cytoplasm "so that each egg appears to possess a considerable number of small nuclei." Lillie concludes from

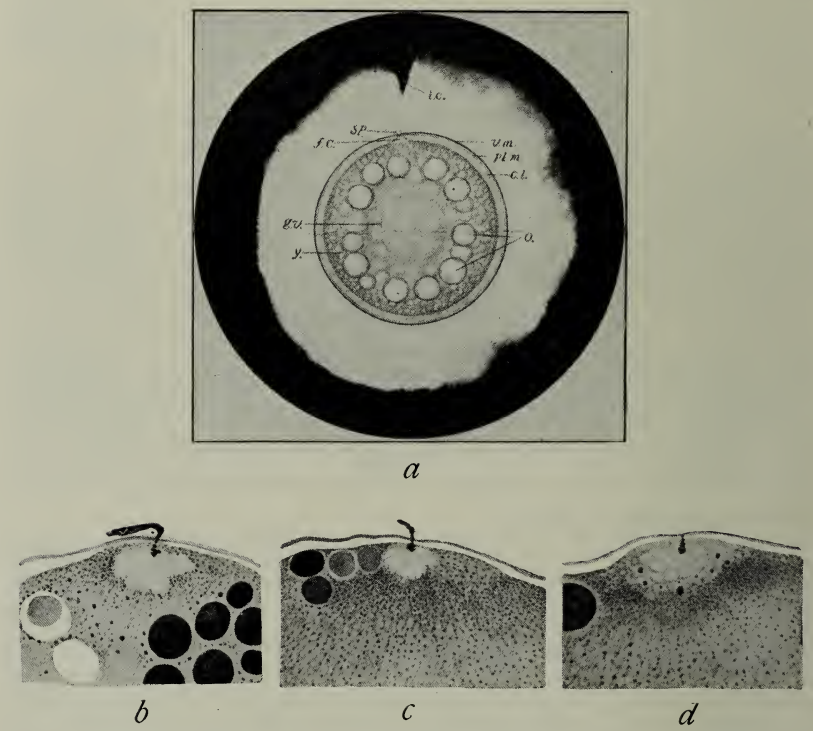


FIG. 6.—Normal fertilization of the egg of Nereis (above). Partial fertilization by means of a piece of the sperm-head (below). (After Lillie.)

a comparison between these partially fertilized and centrifuged eggs, and eggs centrifuged without being affected by the sperm that "the partial stimulus of the spermatozoön is somewhat more effective than the mechanical shock of centrifuging, though both produced the same initial changes, apparently equally well."

These conclusions of Lillie's have been fully confirmed by Goodrich ('20) who, at Chamber's suggestion, removed by means

of a micropipette the first spermatozoön that touched the egg and followed the subsequent changes in the egg. His results show that "the full stimulating effect" is reached in a very few minutes after attachment, "quite possibly it is only a matter of seconds." The egg then proceeds to extrude its jelly and to give off one or both of the polar bodies. Its development does not proceed beyond this point.

Lillie ('12) has also found cases of *fractional fertilization* in the centrifuged eggs of Nereis. It appears that when the elongated head of the sperm is halfway inside the egg, the part lying outside may be broken off by centrifuging. At the broken base of the part that has entered an aster appears. Its size is proportionate to the size of the sperm-piece taken in. It divides, often to form a mitotic spindle. A small nucleus also forms from the piece of the head. The egg-nucleus proceeds to mature, the second polar body is given off and the egg pronucleus is formed. This nucleus and the fragment of the sperm-nucleus meet and fuse, and later the chromosomes may appear and divide. In a few cases the egg divides, and these cases are presumably those in which a complete spindle has formed. In other cases only a monaster appears near the chromosomes, and these eggs do not divide. In other cases still, no aster can be found. It is probable that these differences are connected with the size of the aster that develops as well as with its subsequent stages. Lillie thinks that the aster is formed through the influence of the piece of the sperm-nucleus because the centrosome of the original cell that produced the sperm lies at the base of the tail and has been excluded in this case. Lillie argues that the evidence proves that an aster may develop independently in the vicinity of the sperm.

CHAPTER VII

POLYSPERMY

As a rule only a single spermatozoön enters an egg and fertilizes it. If more enter the result is disastrous. In such eggs the injury due to polyspermy is generally the result of the development of an abnormal mitotic figure (a triaster or tetraster) which brings about an irregular distribution of the chromosomes, and abnormal types of cleavage.

On the other hand, in a considerable number of cases, the mechanism is such that even when several sperms enter, only one sperm-nucleus unites with the egg-nucleus, and a single mitotic spindle develops. The other sperms sooner or later disintegrate. For instance, it was shown by Rückert ('99) that the large eggs of selachians are normally entered by more than one spermatozoön. Only one reaches the egg-nucleus, and, then, as the segmentation spindle develops, the near-lying sperm nuclei are pushed out to the periphery of the blastodisc. Here they may divide, but the resulting nuclei take no part in the formation of the cells of the blastoderm. In a newt, Diemyctylus, Jordan ('93) found that more than one sperm enters the egg without disturbing the normal development and this has been found to hold for other urodeles. In the reptiles also, many sperms have been found in the egg (Oppel '92) that are otherwise normal. In the pigeon, twelve to twenty-five sperms may enter the egg according to Harper ('04). When the second polar body is extruded a ring of sperm is found around the maturation spindle, but at some distance from it. One of these sperms moves centrally to unite with the egg-nucleus, while the others are pushed out to the edge of the blastodisc as the astral figures develop. These rejected sperm are later absorbed according to the observations of Blount ('09).

In Bryozoa many sperms penetrate the immature egg (Bonnevie '07) but only one fertilizes it. In some insects a few sperm

may enter the egg (Henking '91, Huettner '27), but union with the egg-nucleus is with only one of them.

One of the most completely worked out cases of abnormal polyspermy is that of the frog's egg in which normally only one sperm enters. Several may enter simultaneously if the egg is placed in concentrated sperm, or if kept under abnormal conditions. Such eggs break up generally into irregular pieces (baroque segmentation) as observed by Born, Brachet and others. The process has been studied in detail by Brachet ('10, '12) and by Herlant ('11). They find when the eggs are heavily inseminated that several or even many spermatozoa may enter the black hemisphere at the same time (Figs. 7 and 8). Each carries in a centrosome around which an aster rapidly develops. As the asters increase in size, i.e., as the more solidified material of which each is formed increases in volume, they come to share equally the material of the upper hemisphere of the egg. This can be interpreted to mean that the more solid masses push each other away through the more liquid parts of the egg that have not yet solidified. As a result each aster comes to occupy a proportionate part of the egg (Fig. 7*a*). As the asters develop, each contains a sperm-nucleus at or near its center. The egg-nucleus moves into that aster that lies nearest to it, and flattens against the sperm-nucleus, there present, to form a conjugation nucleus. This double nucleus, as well as the sperm-nuclei of the other asters, next proceeds to divide. Each aster divides into two and a mitotic figure develops from each aster. Each sperm-nucleus is resolved into its haploid number of chromosomes; while the conjugation nucleus has the diploid number of chromosomes. The egg starts to divide into as many cleavage planes as there are spindles; each plane passing through the middle of a spindle, some of the resulting blastomeres come to contain as a result of this kind of division two nuclei (Fig. 7*d*, *e*), from two different spindles and also two asters. The chromosomes pass into a resting stage. Preparations then go ahead for the next division.

The simplest cases are those in which two sperms enter; the next simplest, when three enter. Herlant worked out in detail the changes that take place under these conditions. A dispermic egg divides, as a rule, by one cleavage into two equal cells. The position of the two spindles (Fig. 7*c*) accounts for the result, for they lie opposite each other with their long axes parallel.

Each resulting blastomere has two nuclei with an aster near each. A spindle next develops around each nucleus and the spindles take the position shown in Fig. 7*d*. At the next division two vertical furrows appear in each blastomere, each at right angles to the first plane and each cutting through the middle of a spindle. Six cells result as shown in Fig. 7*e*. The two cells to the right have each a haploid nucleus; the two cells to the left have each a single diploid nucleus; while the two middle cells have each one haploid and one diploid nucleus. In subsequent divisions the cells

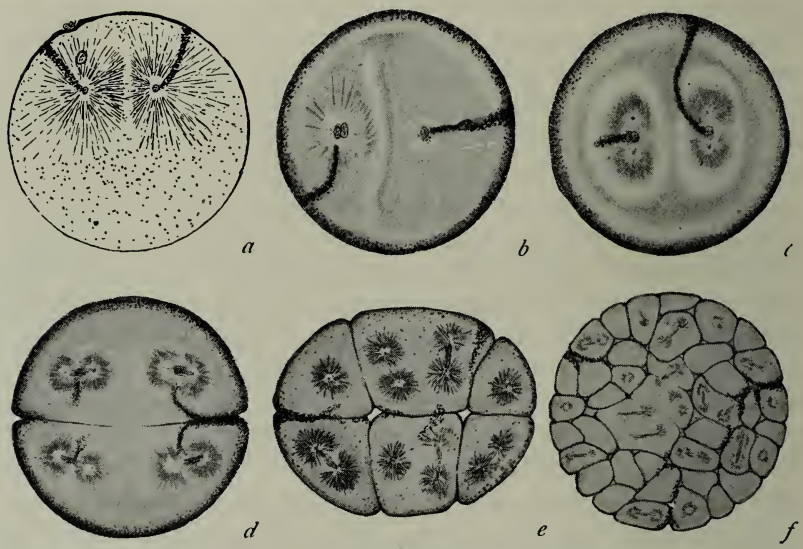


Fig. 7.—Sections of dispermic eggs of the frog. (After Herlant.)

that descend from the single nucleated blastomeres (at the side) will have single nuclei. The descendants of the middle cells will be of two kinds; some of them will have single nuclei—those given off at the periphery; others will have two nuclei, etc. In other words, the double nucleated cells continue to give rise to single nucleated cells, and when a cell has once reached this condition it continues to give rise to single nucleated cells. In the end, unless anomalous types of division come in, most of the cells will have each a single nucleus.

If three sperm enter, their relation to each other, just before division, is shown in Fig. 8*a*. Such an egg divides into three equal or nearly equal cells, each cell with two nuclei (Fig. 8*b*, *c*).

At the next division, each such cell gives rise to two cells, each with a single nucleus, and to one cell with two nuclei. The spindles do not place themselves in a symmetrical position in all the cells, and the surface cleavages are irregular. Nevertheless, the general

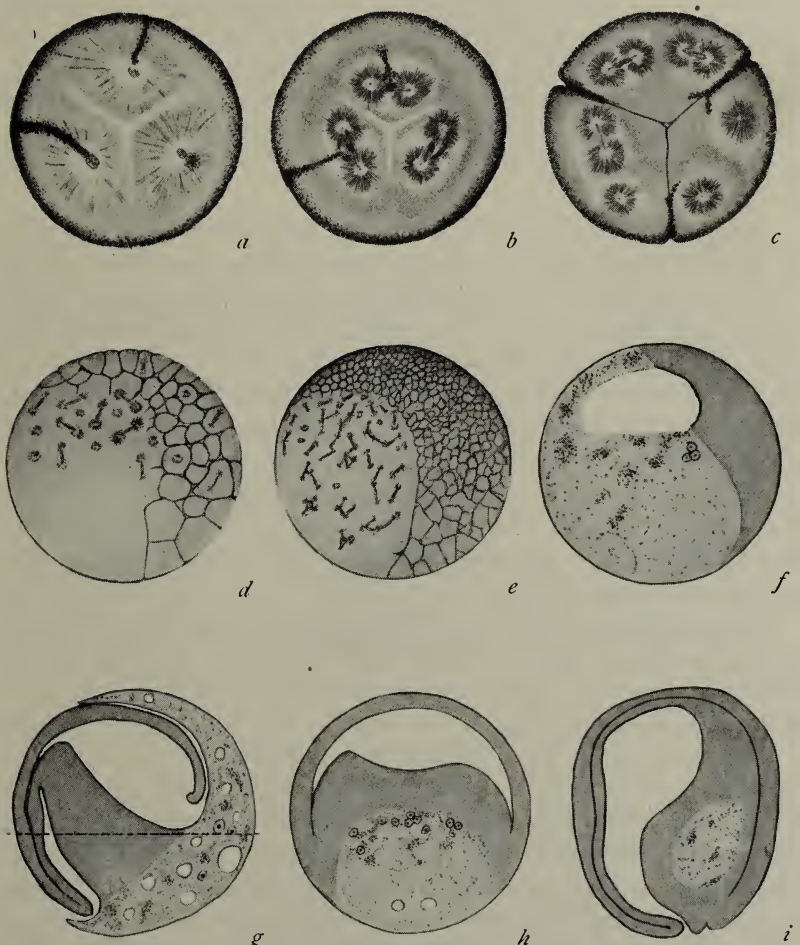


Fig. 8.—Sections of trispermic eggs of the frog. (After Herlant.)

results are the same as in the dispermic eggs of the same stage, although more irregular. Here also single-nucleated cells continue to be produced at the expense of the two-nucleated cells.

Both dispermic and trispermic eggs give rise to blastulae and

gastrulae like those of the normal egg, but they differ from the normal in that they are composed of two kinds of cells, haploid and diploid. Some of the cells also contain more than one nucleus. Furthermore, in many cases there are areas that are not divided up into cells (Fig. 8*d*, *e*). These have arisen by irregularities in the early divisions where cleavage planes have failed to separate the materials completely into cells. A process of this sort, once started, continues because the chance of divisions completing themselves is lessened as the nuclear spindles become smaller, or the mitotic figures come to lie deeper in the mass of common protoplasm.

The embryos that develop show many abnormalities, and even the most normal ones do not live more than a few days. Some of the causes of the abnormalities are apparent, such as the presence of fused areas in the mass, cells of unequal sizes, and cells with haploid and diploid nuclei (Fig. 8*f-i*). The death of the larvae is supposed to be due, in the main, to the last-named, anomalous conditions, because, Herlant thinks, such different kinds of cells (haploid and diploid) could not be expected to function in harmony with each other. This interpretation seems doubtful in the light of genetic evidence. However this may be, it is evident that little is to be expected from eggs that have divided so abnormally and imperfectly.

When more than three sperm enter, the first and later divisions are much more irregular—often only superficial for large parts of the egg, and such eggs fail more often to give even an approach to normal embryos.

In sea-urchin's eggs, polyspermy has long been a familiar occurrence (Hertwig, O. and R., Boveri, Driesch, Morgan, etc.). When two sperms enter simultaneously, both sperm-nuclei unite with the single egg-nucleus. The sperm-asters are so feebly developed before the sperm-nuclei reach the egg-nucleus that they offer no obstacle to such a union. Hence, from the beginning, the results are different from those in the frog's eggs. By division of the two asters of dispermic eggs, four asters are formed around the triploid nucleus (Fig. 9*a*). Not infrequently one of them is pushed so far outside the central area that it fails to form a central spindle (Fig. 9*c*, *d*), and does not, therefore, get any chromosomes. A triaster forms, and the egg divides into three equal parts. When all four centers surround the compound

nucleus, a tetraster forms, and the first cleavage is into four equal cells (Fig. 9*b*).

The subsequent cleavages in these two kinds of eggs, as described by Driesch ('92) and Morgan ('95) are as follows: At the second division of the three-fold type, the blastomeres divide by meridional planes into six cells (Fig. 10c). The next division is equatorial, forming two rings of six cells each (Fig. 10*d*, *e*). At the next division, each of the six antipolar cells gives rise to a micromere, while each of the six polar cells divides equally to

Fig. 9.—Tetrapolar and tripolar spindles of dispermic and polyspermic eggs of the sea-urchin. (After O. and R. Hertwig.)

produce twelve cells (Fig. 10*f*). It is obvious that the planes of cleavage follow the normal sequence, but in a three-fold instead of a two-fold pattern.[1]

In the other case—the four-fold type (Fig. 11*a*)—each of the first four blastomeres divides at the second cleavage in meridional planes (Fig. 11*b*). There follows an equatorial cleavage

[1] Trefoil eggs of Cerebratulus have been reported by Yatsu ('10). The second cleavage is through the pole, giving, as in the sea-urchin, a ring of six cells. The third cleavage is nearly equatorial (as in normal eggs). At the next cleavage the direction of the division is the same as at the normal, fourth cleavage. These trefoil eggs are probably due to dispermy. The fate of these eggs is not stated.

(Fig. 11*c*); and then each cell of the antipolar hemisphere gives off a micromere, while the cells of the polar hemisphere divide equally (Fig. 11*d*). In this type also it is evident that the initial doubling is followed by divisions that conform completely to the normal pattern.

Sometimes the four-fold type divides at first to produce a tetrahedral form as shown in Fig. 11*e*. A sixteen-cell stage from such an egg is represented in Fig. 11*f*.

Swimming blastulae develop from these eggs, but only very rarely a normal embryo. Boveri, who has studied very carefully

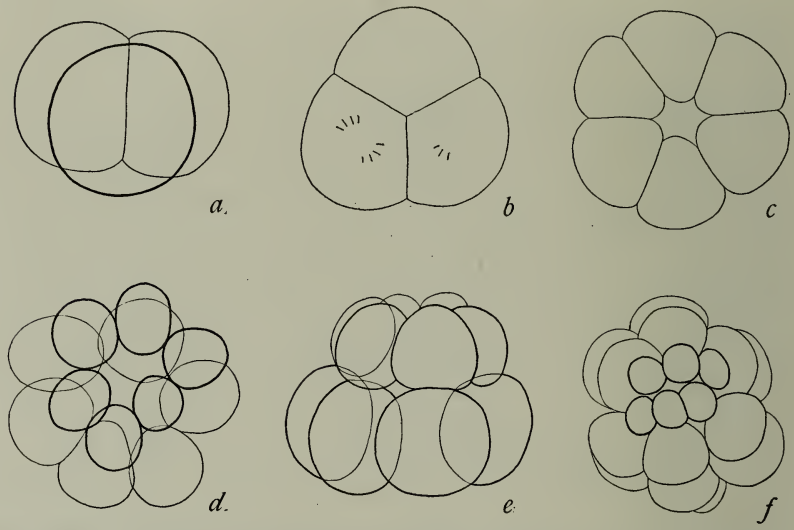


Fig. 10.—Cleavage of dispermic, trefoil egg of sea-urchin. (After Morgan.)

the method of sorting out of the chromosomes in the triaster and tetraster, thinks that it is improbable that any one of the first formed cells ever gets a normal set of chromosomes. The abnormalities that result, he attributes to the absence of a normal set of chromosomes in each cell, and brings forward strong analytical evidence in support of his conclusion.[2]

From the examples it is obvious that the disastrous results of polyspermy in the frog and in the sea-urchin are due to different

[2] Genetic studies have shown that something more than the presence of one set of chromosomes is the significant feature of these divisions; for, even with one of each kind present, the occurrence of others in the group is expected to unbalance the genic relations.

conditions. In the frog the nuclei have each at least one set of chromosomes, but in the sea-urchin this is generally not the case. In the frog, the abnormalities in the later development are not to be referred to absence of sets of chromosomes, but rather to irregularities in the division of part at least of the protoplasm, while in the sea-urchin the division of the protoplasm is strictly regular, but the chromosomal distribution is disturbed. Either situation will endanger the formation of a normal embryo.

The cleavage of the dispermic eggs of Dentalium has recently

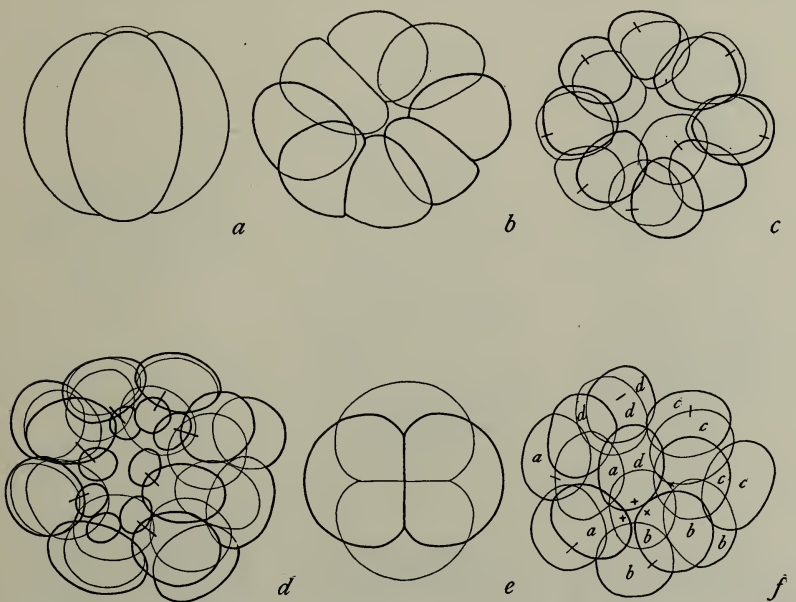


FIG. 11.—Cleavage of dispermic tetrafoil egg of sea-urchin. (After Driesch.)

been described by Schleip ('25). The presence of a "yolk-lobe" in these eggs is a unique feature of the cleavage. The normal cleavage of Dentalium is shown in Fig. 90. Eggs that presumably are dispermic, because they divide at the normal time of the first cleavage into three or four blastomeres (Figs. 12 and 13), are occasionally found amongst normally dividing eggs. Other eggs, presumably polyspermic also, occur that show irregular constrictions.

In the three-fold type, the cleavage divides the egg into three equal or nearly equal parts, but the yolk-lobe field may remain

attached to one, or two, or to all three blastomeres. The blastomere or blastomeres that contain all or a part of the field, are labelled CD, while those without it are labelled AB (Fig. 12).

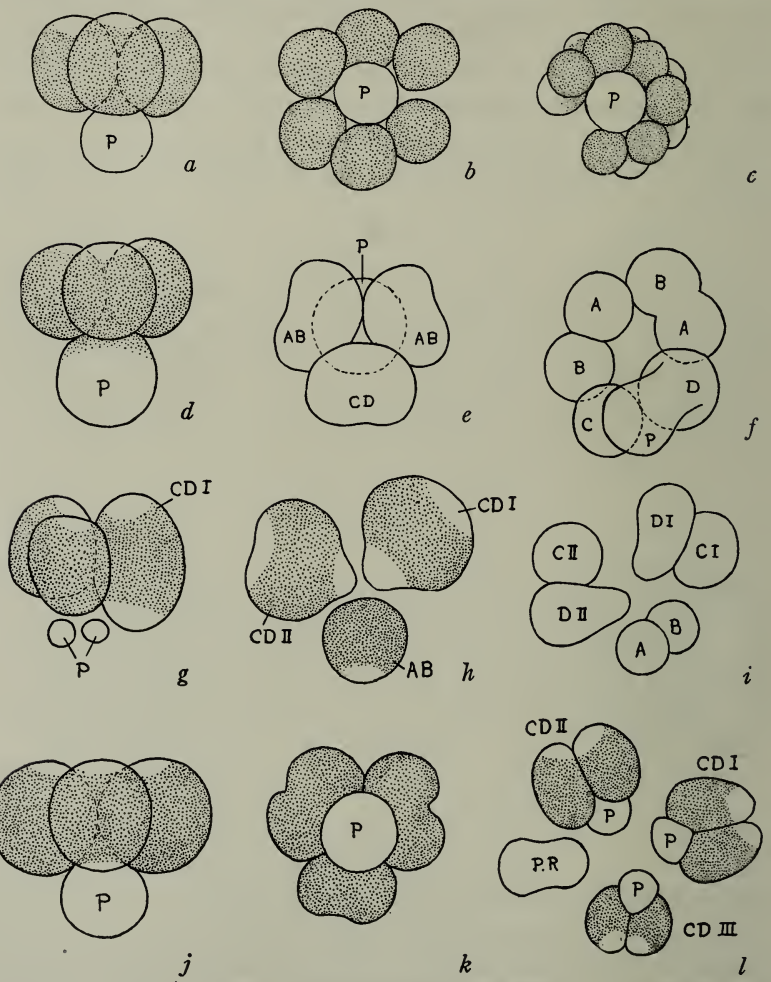


Fig. 12.—Trefoil cleavage of dispermic egg or Dentalium. (After Schleip.)

In Fig. 12*a*, *b*, *c*, the yolk-lobe was cut off entirely from all the blastomeres. The second division was again radial, giving six AB-cells. The third division was dexiotropic. In Fig. 12*d*, *e*, *f*, the yolk-lobe is attached to one of the three blastomeres. In Fig. 12*g*, *h*, *i*, the yolk-lobe field is attached to two blastomeres.

It soon separated off from one of the two. In Fig. 12*j*, *k*, *l*, all three blastomeres contain some of the yolk-lobe field (a piece was also constricted off).

When the egg divides into four cells in one plane (by two meridional divisions) the yolk-lobe may be attached to one or more blastomeres, and at the same time the polar field may be pinched off (Fig. 13*a*, *b*). In a few eggs, the first division produced a tetrahedral arrangement (Fig. 13*c*), the yolk-lobe remaining as part of one blastomere.

It is evident that these dispermic eggs of Dentalium show essentially the same relations as do dispermic eggs of other animals. There is a general tendency for the typical cleavage

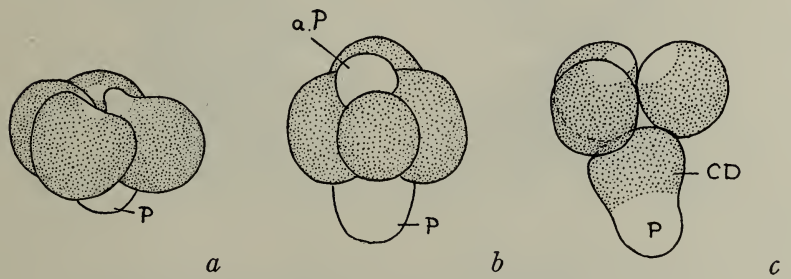


Fig. 13.—Tetrafoil cleavage of dispermic egg of Dentalium. (After Schleip.)

pattern to reproduce itself insofar as compatible with the kind of first division that occurs.[3]

Boveri ('10) has described the cleavage of the eggs of Ascaris that are supposed, on indirect but sufficient evidence, to be dispermic. For comparison, the cleavage of the normal egg is shown in Fig. 14. He recognizes three types of dispermic eggs (Fig. 15*a*, *a'*, *a''*). In the first type there is one cell that behaves like the P_1 cell of the normal egg; in the second type there are two such P_1 cells; and, in the third type, three. Boveri concluded that in the four-fold type of cleavage, the first four cells have the same values as have the first two cells of the normal egg; namely AB or P. There may be three cells having the "qualities" of the AB, or two, or only one, the others having the quality of P_1. Already, in the transition from the four- to the eight-cell stage,

[3] Schaxel ('13) has described the spindles and the cleavage of polyspermic eggs of the polychaetic Annelid, Aricia.

it is possible to tell with certainty from the direction of the divisions which of the primary blastomeres are AB and which P_1. Their values are confirmed by the further fate of the cells. Boveri concludes that the injurious effect of the dispermy in Ascaris is brought about, not as in echinoderms by the irregularities in the division of the chromatin, but by the wrong "plasma qualities" of the cell-complexes. He says that he is convinced that, if a blastomere of the dispermic egg of Ascaris contains at least one

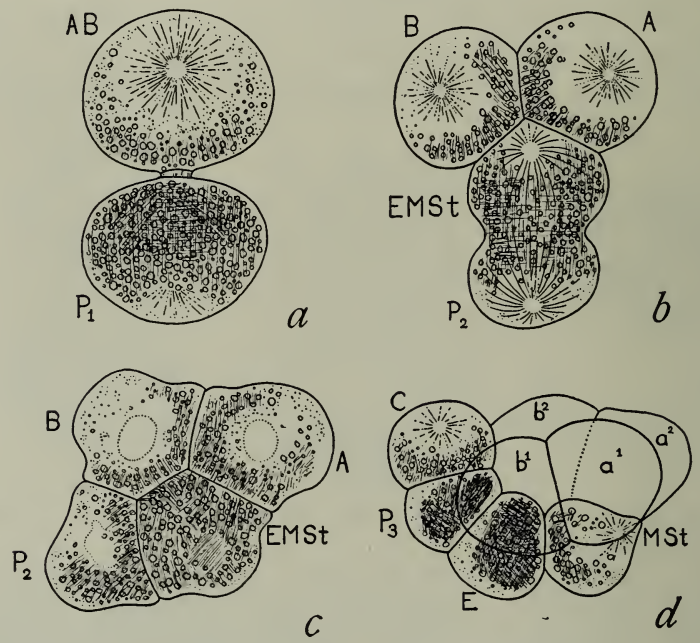


FIG. 14.—First three cleavages of the egg of Ascaris megalocephala. (After Boveri.)

chromosome, it can develop into a definite portion of a normal embryo *providing it finds itself* in the *right* environment (plasma). Expressed more concretely he says: "I do not doubt that if it were possible to separate a dispermic egg of type II at the four-cell stage into two groups, each AB-P_1, or if one could separate, in type I, the cell P_1 with any one of the three cells AB, or if one could isolate the cell AB with one of the three P_1 cells in type III, a normal dwarf-embryo would develop from each such cell-group."

The preceding account relates largely to the descriptive and

analytical work on polyspermy. A few experiments have been made on the eggs of Triton that are normally polyspermic. At the point of entrance of each spermatozoön a dark spot is left for a time on the surface (Fig. 16). If immediately after the sperm has entered, a loop of thread is tied around the egg and then tightened, the egg will be constricted into two parts, one part containing the egg-nucleus with or without a sperm-nucleus, the other with only one or more sperm-nuclei as seen by the dark

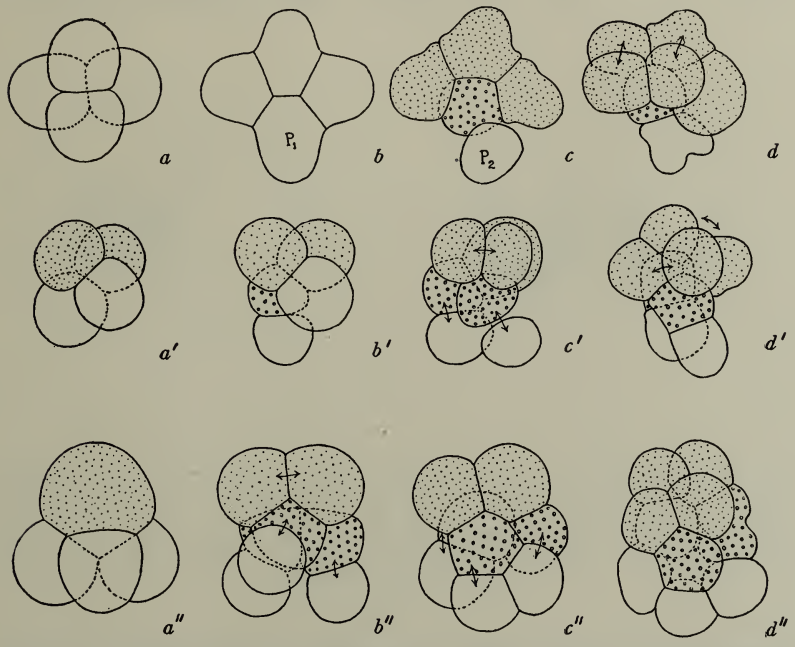
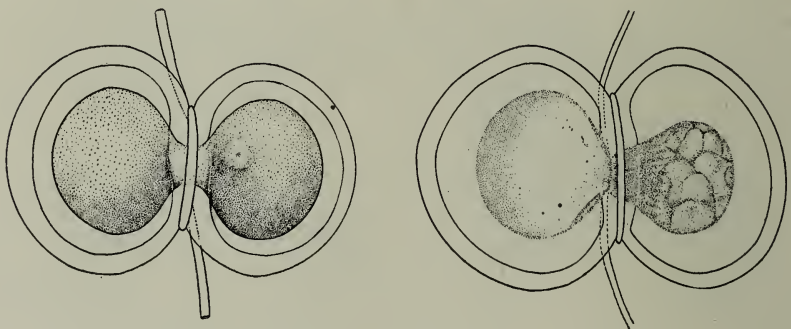


Fig. 15.—Tetrafoil cleavages of the dispermic egg of Ascaris. (After Boveri.)

spots on its surface. The possibilities of haploid development were studied by Spemann ('14, '24) and by Baltzer ('22) by the use of this method and will be described in a later chapter. The behavior of the nuclei in the constricted eggs has been examined by Fankhauser ('24, '25). He reports that both monospermic and polyspermic eggs, with less than ten sperm in them, develop normally—one sperm-nucleus uniting with the egg nucleus. When more spermatozoa enter, the cleavage is abnormal, since both the principal and the accessory sperms may take part in the division. In 92 operations, the egg was constricted in such a way that both

the nucleated and the non-nucleated portions contained spermatozoa. The former developed normally with the suppression of the accessory sperms; the latter, when numerous sperms were present, often divided as do normal eggs, but 87 per cent divided abnormally, due to the independent development of several sperms. The cleavage of the sperm-containing portion is delayed.

In another series of operations, 52 cases were obtained in which one portion of the constricted egg contained only the egg-nucleus and no sperm-nucleus. This portion, with an egg-nucleus, but no



A *B*

Fig. 16.—*A*, partially constricted egg of Triton immediately after fertilization. In the right-hand portion the clear area with a darker center marks the point at which the polar bodies have been given off. The portion to the left might have shown dark spots where spermatozoa have entered. *B*, the same egg; the right-hand portion has begun to cleave, but as yet no cleavage has appeared in the left-hand portion. (After Spemann.)

sperm-nucleus, often began to segment. Frequently it incompletely cleaved, sometimes the cleavage proceeded normally to the morula stage. In one case the parthenogenetic development went so far as to produce an embryo with a closed medullary plate.

In a third series of operations including 228 cases, the eggs were only slightly constricted into a dumb-bell-shaped form. When the connecting bridge is thick, the development of the accessory sperms is suppressed as in the normal egg; when the bridge is narrow the portion that lacks the egg-nucleus may cleave as it does when entirely separated. The suppression is due, Fankhauser thinks, to chemical influences coming from the egg-nucleus across the bridge. Possibly the more rapid development of the portion

that contains the egg-nucleus might account for the result as well as the postulated influence of the egg-nucleus.

If the portion without the egg-nucleus alone contains sperms, both the sperm-nuclei and their sphaeres, as well as the egg-nucleus, begin to develop. In more than 50 per cent of eggs, one of the sperm-nuclei reaches and fuses with the egg-nucleus, the two nuclei meeting in the bridge or in its vicinity. These results demonstrate, Fankhauser suggests, that the nuclei have some influence on each other.

CHAPTER VIII

THE FERTILIZATION MEMBRANE

The egg of the sea-urchin, when set free, has over its surface a thin coat of jelly which quickly absorbs water and swells. This takes place whether the egg is fertilized or not. To this coat of jelly the spermatozoa adhere. In some species of sea-urchins it is firm at first, and not easily displaced (Arbacia); in other species (Strongylocentrotus purpuratus) it is very soft and easily disturbed or lost. Moreover, in some females of S. purpuratus, the jelly is absent (or has been lost) while the eggs are still in the ovary. This may depend, perhaps, on the length of time the egg has been in the ovary, or on the condition of the female. At any rate, I have found that, in females kept for two or three days in sea weed, the jelly is absent from many of the eggs when collected in sea water. Eggs without the jelly are fertilized with difficulty, or fail to be fertilized, as Elder ('12) first observed. Watching such eggs, I have observed that the spermatozoa that run into them fail to stick, hence do not enter, although if one does enter it fertilizes the egg. It may appear, then, that one of the functions of the jelly is to catch the sperm, and to supply enough resistance to help them puncture the outer surface of the egg. As the jelly of the egg swells it becomes difficult to see, but its presence is indicated by the broad ring of active and immobile sperm that forms a halo in its outer layer.

The surface of the unfertilized egg has a sharp, definite boundary. There is some sort of a membrane covering the surface, but it is not conspicuous, partly because its refractive index is about the same as that of sea water. This surface layer is almost instantly changed into the fertilization membrane when it is pierced by a spermatozoön.

The formation of this membrane was first observed in the starfish by Fol in 1876, who recorded that it began to appear first at the penetration point of the sperm. Other and later observers

have described the membrane of the sea-urchin's egg as appearing simultaneously over the whole surface, but more recently Just ('19), who has studied its origin in the larger egg of Echinarachnius, finds that it starts at the entrance point of the sperm as a blister; and thence extends rapidly around the egg, taking only a few seconds to reach the opposite point.

The fertilization membrane is very tough, but it can be shaken off when it is first formed; i.e., within a minute or two after fertilization. This membrane appears to come from the most superficial layer of the egg, but whether its substance represents unchanged the outermost layer of the egg that is simply lifted away from the surface of the egg, or whether its substance is produced by the egg at the time of its appearance, or whether it is only a precipitation reaction, is not certainly known, but has been the subject of much debate and experimentation.

The formation of the fertilization membrane has not attracted the same attention in the eggs of other animals as in those of the sea-urchin, but in most unfertilized eggs there is present on the outer surface a delicate "vitelline" membrane that can be seen in some cases to separate from the surface of the egg at the moment the sperm penetrates the surface. Such a membrane is present in the frog's egg. Immediately after fertilization, fluid appears between the membrane and the surface of the egg. In Nereis there is present a definite membrane around the newly laid egg through which the secretion, that turns into jelly on coming in contact with the sea water, is poured out. A space appears between the membrane and the surface of the egg—a space traversed by radial lines that are said to be the collapsed walls of the superficial layer of droplets that have emptied themselves through the membrane to form the jelly. The egg forms a new surface lying just beneath the former superficial layer of droplets.

In Ascaris the egg appears naked until the sperm enters, when it pours out a jelly that hardens to form a tough impenetrable covering. Later the egg shrinks away from the jelly, and comes to lie in a rather large fluid space.

The loss of substance that takes place when the membrane or the jelly and the perivitelline fluid is formed must mean that the egg becomes correspondingly smaller. Measurements of the sea-urchin's egg by Glaser ('14, '24) show that the egg decreases by

10 per cent. Other observers (Loeb and McClendon) failed to find any measurable shrinking. Okkelberg ('14) found that the volume of the egg of the brook lamprey is reduced 14 per cent.

At the point of penetration of the sperm, a fertilization cone appears (Fig. 6*a*) which forms, for a short time, a very conspicuous feature, especially in stained eggs of annelids, molluscs, sea-urchins, ascidians, etc., where the cone is well developed. It consists apparently of ectosarc that flows to the point of entrance. In Nereis it projects above the general surface into a cone-shaped protuberance. After it has engulfed the head of the spermatozoön, it is withdrawn, and a depressed area of the surface takes its place. The sperm-head, and frequently the entire spermatozoön, passes through the cone into the deeper layers of the egg. The actual rôle that the fertilization-cone plays in engulfing the spermatozoön is not understood. Rhumbler thinks that it involves a surface tension reaction.

In some eggs, only the head of the spermatozoön enters (Nereis); in others the head and middle piece enter (sea-urchin, frog); in still other cases the whole spermatozoön may pass into the egg (Physa, Ascaris, crustaceans, some insects, amphibia and mammals). The centrosome of the middle piece of the spermatozoön passes in with the head; the rest of the middle piece is later absorbed (sea-urchin), as well as the tail, if it has entered.

After the fertilization membrane has lifted from the surface there appears over the egg a very thin clear outer layer, sometimes called the ectoplasm, sometimes the hyaloplasm-membrane. It becomes especially conspicuous during the first and second cleavages where it is seen to follow the cleavage furrows between the cells. It has every appearance of being a "living" part of the cell.

Several observers have recorded at times the development of a second membrane under the first one (Herbst, Loeb) that is also lifted from the egg. This is a very significant fact, since it indicates that the surface of the egg, when freed from its first membrane may, under peculiar conditions, form a second one that has the same property of becoming lifted off the egg. It also appears that a membrane can form over small fragments broken from the unfertilized eggs, if these eggs are fertilized (Hertwig, O. and R., Morgan, Ziegler, Chambers). In such cases, it is practically certain that the membrane of the fragment is derived

from the original surface layer of the egg that covers a part, at least, of the fragment. Chambers has shown that fragments composed of endoplasm (i.e., without any of the original surface) cannot be fertilized, and do not form membranes, but if a part of the ectoplasm remains, a spermatozoön may enter in this region. A membrane is formed only over the surface that is covered by the ectoplasm.

Heilbrunn ('13, '15) has made a detailed study of the origin and composition of the fertilization membrane of the sea-urchin's egg. He regards it as a vitelline membrane comparable to that found in many other eggs. It is present before fertilization, but becomes more visible when lifted from the surface of the egg after fertilization. It does not, according to Heilbrunn, result from a reaction between sperm and egg, although soon after it is lifted off it may change its composition to the extent of becoming more permeable.

Careful examination of the living, unfertilized eggs of the sea-urchin reveals a thin membrane over the surface. If the egg is burst, and its contents squeezed out, the membrane is left behind, as Herbst ('03) first demonstrated. Dilute acids cause it to swell. In a solution of one part normal HCl plus nine parts sea water, the membrane swells and becomes sticky. The eggs may then adhere to one another. Dilute solutions of nitric, butyric, and valerianic acids also cause the membrane to swell, but it does not pass into complete solution. Dilute alkaline solutions dissolve it away without causing any swelling or very little. Unfertilized eggs in an alkaline solution also become sticky, and adhere to the dish or to each other. The surface of the egg becomes rough when the membrane is dissolved, but does not go into solution because its surface is coagulated. The behavior of the membrane toward acids, alkalis, and salts indicates its protein nature. That it is not purely lipoid is shown by the fact that it is not soluble in any of the lipoid solvents. Nevertheless it may be a mixture of lipoids and proteins, but Heilbrunn thinks this improbable because of its infractive index which is more like that of some proteins than like that of lipoids and because it does not stain in Scharlach R as expected if it contains lipoids.

The membrane is lifted from the egg by an accumulation of fluid beneath it. The membrane itself does not become any thicker. After elevation it shows nearly the same chemical properties

as before fertilization. After it has been lifted off a new layer appears over the protoplasm. This is called the hyaline layer, as mentioned above. It adheres to, or is a part of, the surface of the egg and during cleavage follows the cleavage planes between the cells.

The fertilization membrane is permeable to electrolytes, and, since it offers considerable resistance to their passage before elevation, it must undergo a change in permeability after or during elevation. This change has, in fact, been suggested as the cause of its elevation, but Heilbrunn has shown that the change in permeability does not take place until the elevation is about complete.

That the formation of the membrane is not due to the swelling of a colloidal substance at the surface of the egg is shown, according to Heilbrunn, by the following evidence.

1. All the reagents that cause membrane elevation cannot be supposed to induce the swelling of colloids, such reagents as distilled water, alcohol, chloroform, picric acid.

2. The elevated membrane does not swell, on the contrary it becomes more rigid. Swelling is correlated with a decrease in rigidity.

3. The membrane-producing reagents do not produce liquefaction but have an exactly opposite effect on colloids of the sea-urchin's egg. Membrane formation cannot be said to be due to colloidal swelling and liquefaction and at the same time be described as cytolytic, for cytolysis results in coagulation.

Heilbrunn brings forward experimental evidence, which, he argues, goes to show that artificial membrane elevation is "apparently" always the result of lowered surface tension of the vitelline membrane. It is probable, therefore, that the sperm produces the same effect, due either to some substance that it carries, or to the physical effect of penetration of the membrane. The first alternative is improbable because no such substance can be extracted from the sperm although concentrated sperm may cause the vitelline membrane to swell. If, on the other hand, the tension of a stretched film or membrane be lowered at one point there will be a lowering of the tension at every other point. The sperm might do this, by simply piercing the membrane. If it be objected to this interpretation that a needle prick should give the same effect, it may be said that unless the hole were at the

same time plugged up the change in tension would not result for sea water would enter through the hole.

More recently Heilbrunn ('24) has made a further study of the many kinds of chemical and physical agents that cause membrane-formation, and reaffirms his earlier conclusion "that every known method of producing membrane elevation results in a lowering of the surface tension of the liquid surrounding the egg," thus causing a lowering of tension of the outer covering of the egg. It is not possible to give here in detail Heilbrunn's discussion of the application of this generalization to all cases, but a few illustrations may suffice. Some of the substances that Heilbrunn described in his earlier paper as causing membrane formation, he found later caused only a softening or swelling of the surface. This effect, he thinks, is to be sharply distinguished from true membrane formation. He states also that the effects of metallic copper and of silver used by Herbst ('04) and by Mathews ('07) to cause membrane elevation only produce swelling. The silver coins that were used to produce membranes are alloys of copper. Copper is attacked by NaCl in the presence of air, and the solution becomes alkaline, oxychloride being formed. The effect becomes, therefore, only a special case of the action of alkalis.

In contradiction to Heilbrunn's conclusion, that the elevation of the fertilization membrane of the sea-urchin egg is due to a lowering of surface tension, Just ('22) has pointed out that the membrane may be lifted in *hypertonic* solutions of KCl and of NaCl in sea water. It is true that a long sojourn (fifteen minutes) in the hypertonic solution is necessary to produce this effect, but since normal, top-swimming plutei result there are at present no grounds for distinguishing this effect of hypertonic solutions from that brought about in other ways. Bataillon ('26) has repeated and confirmed Just's observations on four other species of sea-urchin and has reached the same conclusion, namely, that the result is inconsistent with the assumption of a lowered surface tension.

Heilbrunn ('24) has also studied the egg-membrane of the mollusc, Cumingia. He describes it as a stiff membrane, one micron in thickness. It "governs osmotic intercourse and is therefore a plasma-membrane." In hypertonic solutions the eggs shrink only slightly because of the "stiffness" of the membrane. But if the membrane is caused to swell, then the eggs shrink in hypertonic

solutions. The membrane swells in dilute acids and alkalis, and in NaCl and NaI. It behaves like a protein gel. It does not contain any large admixture of lipoids as shown by a test with Scharlach R.

Two kinds of cortical changes are possible; the membrane may swell or it may be elevated. After fertilization it does not become elevated in Cumingia but swells. Any reagent that causes either of those changes results in the throwing off of the polar bodies. The polar bodies will also be given off if the membrane is ruptured either by shaking or by bursting in a dilute solution. Eggs whose development is started in any of these ways do not often segment. The removal of the inhibition caused by the rigidity of the membrane does not suffice to introduce other kinds of changes essential to the following stages, or else the impulse to polar body formation is not strong enough to lead to further changes.

Loeb had shown ('13, '15) that if the fertilization membrane called forth by butyric acid is shaken off, spermatozoa will penetrate the eggs. New membranes are then formed and normal development follows. But Just ('19) has shown that the entrance of the spermatozoa in such cases occurs only if the action of the butyric acid has not produced the optimum effects possible with this reagent. He finds that those sets of eggs of Echinarachnius in which 100 per cent have produced membranes will, if treated subsequently with hypertonic salt solution, produce large numbers of top-swimming larvae. Now if the 100 per cent butyric acid eggs are removed from butyric acid, their membranes shaken off and sperm added in sea water the sperm do not enter. In other words, if the membrane formation (cortical activation) is complete an irreversible effect has been brought about and the sperm cannot enter the egg. Eggs completely activated by butyric acid have ceased to produce fertilizin. Lillie ('14) and Carl Moore ('16) also reached the same conclusion for the eggs of Arbacia treated with this acid. The contradiction between these results and those of Loeb are shown to be due to the incomplete action of the butyric acid treatment in the egg examined by Loeb. In fact, Just has shown that if the treatment is below the optimum such eggs may, after removal of the membrane, be fertilized by sperm; they then form membranes, cleave, and produce larvae. Even eggs that have been overtreated may be fertilized although

their subsequent development is abnormal. The latter do not form secondary membranes on fertilization. These over-exposed eggs also produce fertilizin until fertilized. Many of the eggs die by cytolysis, but this change is not apparent in eggs with optimum exposure. Just ('23) regards cytolysis as exceptional, therefore, and not a characteristic change of normal or artificial fertilization. He has shown that if the membranes are removed from fertilized eggs of the sand dollar and fresh spermatozoa are added they do not enter the eggs. Their absence was demonstrated by making sections of the eggs. Fragments of fertilized eggs of the sand dollar do not receive the sperm although, as Hertwig, Boveri, and Morgan have shown, fragments of unfertilized eggs will receive spermatozoa. Similar observations (Wilson '03) have been made on egg fragments of the fertilized eggs of Cerebratulus.

By means of a very simple experiment R. S. Lillie ('16, '17, '18), has shown that the permeability of the fertilized sea-urchin

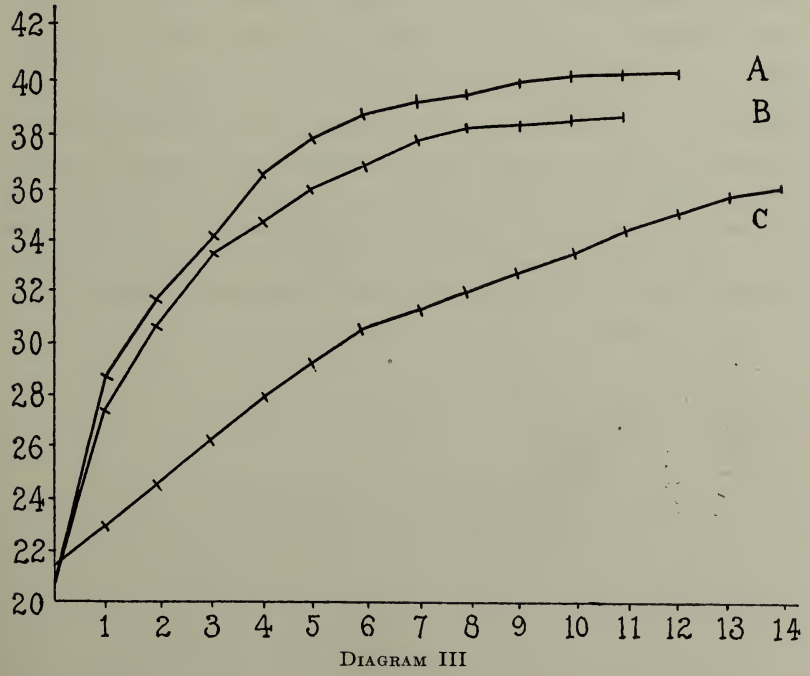


Diagram III

egg is four times greater than that of the unfertilized egg. Arbacia eggs are fertilized, washed to remove excess of sperm, and mixed with equal numbers of unfertilized eggs. They are then

transferred to hypertonic sea water (400 vol. + 60 vol. tap water) and measured under the microscope. In two or three minutes a difference in size of the two kinds of eggs becomes apparent—the fertilized being larger (unfertilized 78μ, fertilized 85μ). The normal egg measures 74μ. The permeability is four times greater in the fertilized than in the unfertilized eggs. The difference in the rate of absorption in fertilized eggs (A), artificially fertilized eggs (B) and unfertilized eggs (C) is shown in Diagram III. The change in permeability reaches its final stage in about 20 minutes. The change is arrested by potassium cyanide (M $\frac{1}{100}$ to M $\frac{1}{400}$). Anesthetics also prevent increase of permeability in concentrations that are similar to, but in some cases higher than, the concentrations arresting cleavage. The effect is readily reversible.

Clowes and Smith ('23) and Smith and Clowes ('24) have studied the influence of hydrogen-ion concentration on the fertilization and development of the eggs of the sea-urchin, starfish and Chaetopterus. When the eggs are inseminated in CO_2-free sea water of varying H-ion concentrations, a block to fertilization appears for Arbacia at pH 6.8, for Asterias at pH 7.0, and for Chaetopterus at pH 7.1. The unfertilized eggs retain for the longest time their capacity to be fertilized and to divide at about pH 6.0. In Arbacia the velocity of division is reduced to 50 per cent of the velocity in sea water (pH 8.15) at pH 5.2 and 9.4. Between pH 5.8 and 8.2 the divisions are normal in velocity. Between pH 8.2 and 9.2 the velocity of division is increased from 15 to 25 per cent.

CHAPTER IX

PHYSICAL AND CHEMICAL CHANGES IN THE EGG AFTER FERTILIZATION

Physical Changes

It has been noticed by several observers that the viscosity of the egg changes soon after it is fertilized and also at definite stages during each cleavage. Such changes are indicated (1) by the greater ease with which the egg may be shaken to pieces at certain stages, (2) by amoeboid changes in its surface, (3) greater or less difficulty in puncturing, and (4) by the effect of centrifuging. Heilbrunn ('15, '17, '21, '26) has taken advantage of the last method to examine in detail the viscosity of the egg at short intervals between fertilization and the first cleavage. The centrifugal force drives the heavier yolk and pigment granules towards the outer pole and the fat to the inner pole, leaving a clearer band between the two. The width of this band serves as a measure of the viscosity of the egg, taken as a whole. The more fluid the substratum, in which the yolk and pigment are imbedded, the more easily will they be driven through it by the centrifugal force; and, conversely, the more viscid, or semi-solid the substratum the slower will be their progress. The depth of the clear region or band should give, therefore, an approximate measure of the viscosity provided the centrifuging has been the same in all cases.

The eggs of the sea-urchin, Arbacia, were placed in small glass tubes and revolved on a hand centrifuge. Each turn of the handle gave 130 revolutions. The centrifugal force is given by the formula $C = \frac{mv^2}{r}$. The mass, m, is unknown, but probably constant, the velocity $v = 2\ \pi\ r$ times the number of turns per second. The radius, r, was 6 centimeters. The eggs were centrifuged about every four minutes after fertilization until the first cleavage was just coming on.

The results show that ten or fifteen minutes after fertilization the protoplasm has begun to be less fluid. At about twenty minutes it has so far hardened that the clear zone did not appear at all or only showed to a slight extent. Heilbrunn calculates that at this time the viscosity has increased "two-fold" at least. "Such a marked viscosity increase is beyond much doubt due to a gelation of the cytoplasm." The gelation reaches its height just prior to the time when the early spindle first becomes visible. As the spindle develops there follows a decrease in viscosity. When the egg is about to divide the viscosity has returned to the starting point. During the stages preparatory to the second division a similar series of stages is passed through.

The change that stiffens the protoplasm is intimately associated with the development of the mitotic figure in the egg. Confirmation of this view is found in several directions. For instance, there are a number of substances that suppress the formation of the spindle in the egg without injuring it; such as ether, chloroform, aceton, paraldehyde, propyl alcohol, chloral hydrate, etc. These substances prevent also the gelation of the protoplasm after fertilization, as shown by centrifuging eggs that have been treated with them. It had been shown by O. Hertwig that low temperatures prevent the appearance of asters and spindles in the eggs. Heilbrunn showed by centrifuging that cold also has a liquefying action on the egg at a time when it is most resistant to centrifuging (from 16 to 30 minutes after fertilization).

It had been shown that hypertonic salt-solutions cause asters to appear in the egg. The centrifuge shows that such solutions cause an intensification of the gelation of the egg.

The action of KCN is not so clear, but it appears to prevent a reversal of the normal gelation of the protoplasm, as long as present, as is shown by centrifuging. It acts also in intensifying gelation.

From this evidence Heilbrunn concludes that the appearance of the mitotic figure is necessarily preceded by or concomitant with a cytoplasmic gelation. The fact that the artificial production of asters in the egg can best be initiated by hypertonic solutions suggests that in the normal egg the formation of the astral rays and spindles is initiated by the abstraction of water from the protoplasm by the growing pronuclei. The gelation produced

by hypertonic solutions can be shown to behave like the normal gelation. When the protoplasm of the unfertilized eggs is gelatinized by hypertonic solutions such gelation can be reversed by ether. "On the other hand, ether has no effect in reversing or antagonizing the gelatinizing (or coagulation effect) of acids or of distilled water. Hence of these three types of gelation, that produced by hypertonic solutions behaves most nearly like the normal."

The viscosity of the egg of Cumingia at different temperatures has been examined by Heilbrunn ('26). The results are shown

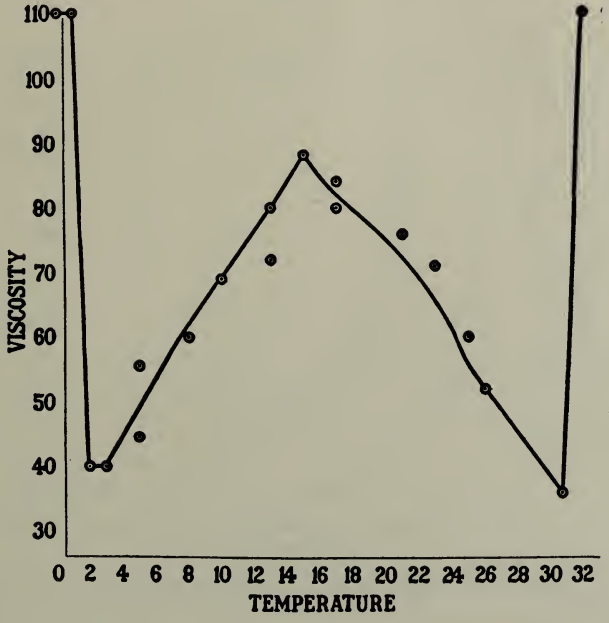


Fig. 17.—Viscosity curve of the egg of Cumingia at various temperatures. (After Heilbrunn.)

in Fig. 17. The viscosity reaches a maximum at 15 degrees C. The coagulation of the egg of Cumingia by heat (Fig. 18) has also been studied by Heilbrunn. He calls attention to the fact that heat coagulation of protoplasm occurs at a lower temperature than that typical for heat coagulation of proteins. Moreover, the coagulation of protoplasm is for a time at least reversible, while that of protein is completely irreversible. The difference, he suggests, may be due to an alteration in the fatty constituents

of the cell, since these can be seen under the microscope to be affected as the temperature is raised to about the point of heat coagulation,—then tend to dissolve; moreover, in the presence of a small percentage of ether the coagulation of the protoplasm may be hastened. "There is other evidence besides that of the effect of temperature to indicate that the fatty constituents of

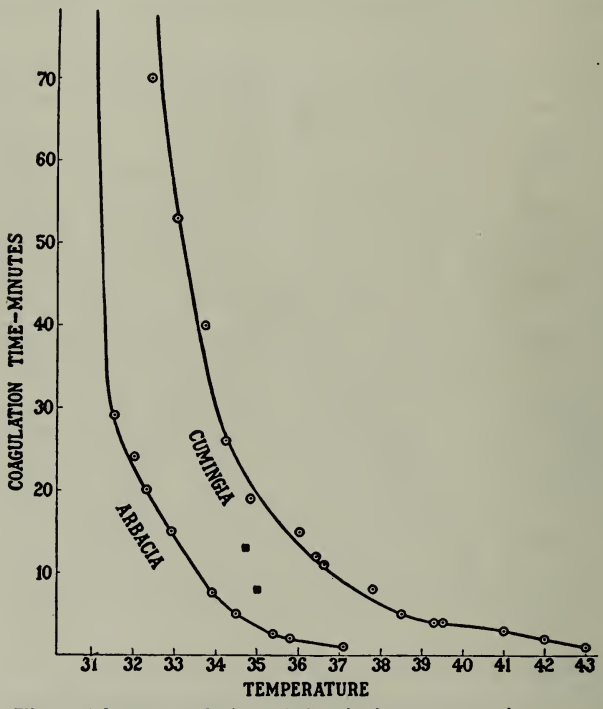


Fig. 18.—Time of heat-coagulation of Cumingia egg at various temperatures. (After Heilbrunn.)

the cell are of great importance in determining both the physical and the biological behavior of the protoplasm. Most fat solvents have a remarkable effect on living things; they stop various manifestations of life without causing death. In other words, they have the power of reversibly inhibiting living processes." The effect of 2½ and 3 per cent ether on unfertilized sea-urchin eggs is shown in Table IX. The third column gives the viscosity of the etherized eggs which may be compared with that of the control eggs in the fourth column. The viscosity of the egg in either is reduced to about one-half its original value.

These experiments can leave little doubt but that the changes following fertilization are intimately connected with the semi-solidification and subsequent liquefaction of the egg. The changes are synchronous with the development of the mitotic figure in the egg, and the two would seem, therefore, to be either the same or parallel changes. The evidence from centrifuging does not show in detail how the two phenomena are related, but another method, micro-dissection, supplies some of the details that are needed to complete the picture.

TABLE IX

Per Cent Ether	Exposure, Minutes	"Viscosity" Etherized Eggs	"Viscosity" Control Eggs	Temperature, C.
2.5	11	10	25	23
2.5	4	15	25	
2.5	8	15	25	22
2.5	10	15	28	24
3	15	15	35	22
3	8.5	20	40	23
3	3	15	30	25.3

The studies of the egg that Chambers ('17) has carried out by means of micro-dissection have given some information relating to the physical condition of different parts of the egg during the stages following the entrance of the sperm. The egg to be examined is suspended in a small drop of sea water on the under surface of an inverted glass slide. The glass needle, whose point is to be pushed into the egg, is made by drawing out a hard glass tube in a small hot flame. The needle, whose end is bent at right angles, is held in a holder connected with an apparatus that can be moved by delicate screws to any desired position. All operations are carried out under a microscope.

The following terms will be used in describing the results. The continuous ground substance of the protoplasm is called hyaloplasm. In it are imbedded granules (microsomes and macrosomes). Although under ordinary microscopic examination the hyaloplasm appears homogeneous, its heterogeneous structure can be demonstrated by illumination from one side. "The presence of minute particles large enough to scatter the light rays are then revealed through the production of a cone-shaped beam

of light known as the Faraday or Tyndal phenomenon. If the particles are not resolvable (in which case they are known as amicrons) a hazy light is all that can be seen. If, however, the particles are large enough (in which case they are known as submicrons) they appear to the observer as shining spots dispersed throughout a transparent medium. In the liquid hyaloplasm, these particles exhibit active Brownian movements." The protoplasm appears to be an emulsion colloid. To the same class belong all the colloids obtainable from organic matter, such as gelatin, glue, albumen and starch. The colloidal protoplasm may exist in two phases, a less viscid sol or a more viscid or firmer gel, and may be transformed from either of these states into the other. This is spoken of as a reversible reaction. "The hyaloplasm of the resting egg is in the sol stage and is of such a slight viscosity that the nucleus and the cell granules can be readily rolled and pushed about in it by the needle. At the egg surface the hyaloplasm is in the gel state, and there seems to be no doubt that the protoplasm owes its high viscosity, extensibility and contractility to the gelatinized condition of the surface film." Within two or three minutes after the sperm has entered the egg of Echinarachnius a tiny aster appears that grows rapidly in size. From the beginning it contains a liquid (sol) center; but the protoplasm around this center has become fairly solid as shown by the fact that the aster may be pushed about by the needle in the surounding liquid protoplasm. The sperm-nucleus is held in the gel around the sphere and may be dragged about with the aster when it is displaced. The aster slowly moves to the center of the egg. Chambers suggests that this movement may be caused by the growth of the gelatinized sphere that pushes the center away from the nearest surface. As the liquid center of the aster increases in size the nucleus moves into it. The nucleus may be dragged out of the center, but so long as it lies within the confines of the aster it will move back to the center. As long as the egg nucleus is beyond the confines of the aster, i.e., so long as it lies in the liquid protoplasm it is stationary.

When the extending rays of the aster reach the egg-nucleus they enclose it, and this nucleus travels toward the center of the sphere where it meets the sperm-nucleus. The movement of the two nuclei to the center of the aster can be explained, he suggests, by assuming that there is an active centripetal current of the

fluid of the rays. These rays are probably also liquid (i.e., they are in the sol state) and are contributory streams to the enlarging center. The substance between the rays is more solid (gel). It is densest nearer the center and more fluid as it extends out into the protoplasm.

The large fusion-nucleus lies in an eccentric position in the central area of the aster (Fig. 19*a*). It appears as though imbedded in the liquid sphere. The rays soon begin to disappear, beginning at what will later be the equator of the dividing egg. This is due to a reversal from the gel to the sol stage, as shown by the movement of the needle through the region that no longer carries distorted threads in its wake. A few rays remain at the two poles (Fig. 19*b*). This stage is very brief. The rays soon

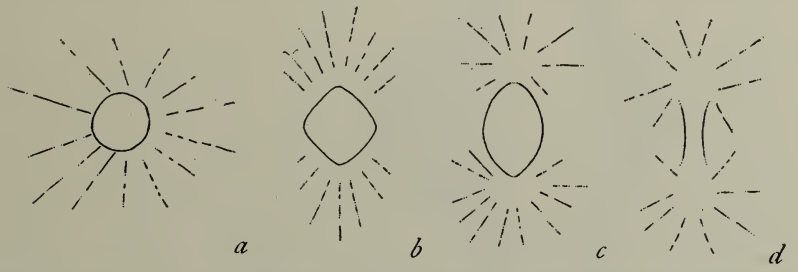


FIG. 19.—Stages in the formation of the spindle in the living egg of the starfish. (After Chambers.)

blaze out again around the two poles. They extend almost to the periphery of the egg and the needle shows that the protoplasm is again a comparatively rigid gel (Fig. 19*c* and Fig. 20*a*). Later a reversal to the sol stage sets in again at the equator, and a flow of granules moves inwards in the equatorial plane of the "spindle." Following this a constriction in the equatorial plane of the cell takes place (Fig. 20*b*) and "one gains the impression that this is consequent to the liquefaction of the cytoplasm in the equatorial plane, while elsewhere it remains in the gel state." During the remainder of this division the rays remain only at the ends of the pole away from the center where the gel stage may be supposed still to persist. When cell-division is completed all the protoplasm has returned to the sol stage. The same process is repeated each time a blastomere divides.

When the amphiaster of the egg is at its maximum stage of development it may be dragged about as a whole in the egg by

the needle. On removing the needle it regains its original state. The rays may be pulled out or bent or the whole figure may be given a spiral twist. This result is possible because of the consistency of the gel between or rather around the more fluid rays. The latter conform to the bending or twisting of the more solid parts. Extensive tearing of the aster may cause reversal, the entire cell passing from a gel to a sol stage.

The physical condition of the egg of Cerebratulus at the time when the polar bodies are forming has also been studied by

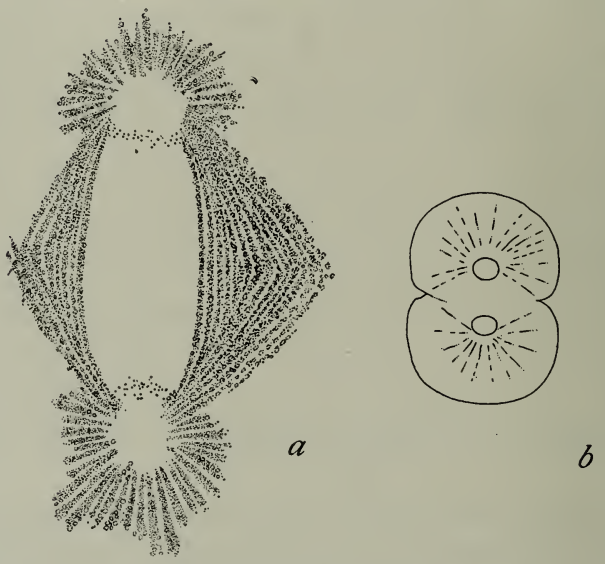


FIG. 20.—Spindle of the egg of starfish at its maximum development, as seen in the living egg. (After Chambers.)

Chambers. When the egg-nucleus breaks down, its place is occupied by a clear area in the granular protoplasm. The clear area "flows" towards the pole. In it appears the first maturation spindle. As it elongates hyaloplasm collects around the spindle poles, above which the rays (gel) become more distinct. In this stage the whole structure may be pushed or pulled about by the needle, or the rays twisted into a spiral. As the gelatinized rays reach the gelatinous periphery of the egg the two form a continuous substance. "Division of the nuclear spindle is followed by a reversal of the astral structure to the sol stage. The sol state of the cytoplasm lying immediately under the surface where the polar bodies are to form is so liquid that granules pushing

into it exhibit active Brownian movement. On the surface, at the middle of this liquid region, the egg bulges forming a nipple-like protuberance. This occurs in such a way as to give one the impression that the protuberance is due to a local weakening in the consistency of the surface where an internal pressure causes cytoplasm to flow out. As one of the daughter nuclei of the first maturation figure lies directly under this spot, it is carried into the protuberance which pinches off to form the first polar body. By pricking with the needle one can so affect this surface as to cause it to produce in succession five or six or even ten protuberances, each being pinched off in its turn and very closely simulating polar bodies. The other daughter nucleus of the first maturation figure now lies free in the protoplasm, and can be pushed about anywhere in the egg. If it be moved out of the hyaline area into the granular cytoplasm, the granules immediately surrounding it gradually move away until the nucleus comes to lie again in a hyaline area. Granules, in the meantime, invade the original hyaline area until it is indistinguishable from the rest of the granular cytoplasm. The nucleus now elongates and migrates again towards the periphery of the egg; astral rays reappear about its poles and, when the second maturation spindle is fully formed, one finds it again firmly attached to the surface of the egg. The second polar body is thus produced some distance away from the first one."

By the micro-dissection method, Chambers has extended and confirmed certain views of cytologists, whose evidence rested, however, only on fixed and stained materials. By entering the living egg it is possible more directly to examine the condition of its various regions, and to discover which of the many proposals that have been made by cytologists come nearest to conditions in the living egg. On the other hand, the method of exploration by means of fine needles is open to the serious objection that the conclusions reached are not as objective as is to be desired, but open at all times to personal interpretations as to what seems to be happening when the end of the needle moves about in the egg. Moreover, there is a further question as to the direct effect of injury to the egg, since the sea water may enter with the needle and cause changes in the egg. It may be added, however, that with proper precaution such injurious effects seem to some extent to be avoided.

Chemical Changes Following Fertilization

In addition to the physical changes and rearrangements of materials that take place in the egg after fertilization, certain changes of a chemical nature are going on at the same time. The consumption of oxygen by the sea-urchin's egg (Arbacia pustulosa) increases suddenly from five to seven times above that of the unfertilized egg (Warburg '08, '10). In another species, Arbacia punctulata, Loeb and Wasteneys ('11, '13) found an increase of four or five times, and in Strongylocentrotus purpuratus five to seven times.

That this change in oxidation is probably connected with the condition of the fertilization membrane at different times was shown by Loeb and Wasteneys in the following way. If unfertilized eggs are comminuted, the particles show a greater amount of oxygen consumption than does an equal amount of *intact* unfertilized eggs, but no more than does the comminuted material of fertilized eggs. They concluded that the change after fertilization is not due to a change in the material of the egg, but to a change in the membrane that permits the absorption of a greater amount of oxygen.

Warburg ('08) carried out a series of carefully planned experiments on the eggs of Arbacia pustulosa, to determine the oxygen consumption before and after fertilization.[1] The following table (Table X) gives the values of four determinations for

TABLE X

Unfertilized Eggs

Oxygen in ccm. thiosulphate; nitrogen in ccm. $n/10$–NH_3

Number	Time	Oxygen in 180 ccm. Water		Calculated Decrease of Oxygen	Oxygen Combined by 28 mg. *N* per Hour
		Before the Experiment	After the Experiment		
14.6	180 min.	16.0	14.9	1.6	0.7
22.1	90 min.	15.5	14.7	1.1	0.7
42.0	90 min.	15.9	14.6	1.9	0.6
29.5	90 min.	15.6	14.7	1.3	0.6

[1] The oxygen was determined by Winkler's method. The eggs were not counted, but the nitrogen was estimated by the Kjeldahl method and the oxygen value stated in terms of quantity of nitrogen.

unfertilized eggs. The results show that 28 mg. of nitrogen take up 0.06 mg. of oxygen per hour.

Fertilized eggs were tested in the same way. The eggs had not segmented at the beginning of the experiment, or else were in the two-cell stage, according to whether the preliminary washing (that took one hour) had been done with cold water or water at room temperature. The results show (Table XI), that after

TABLE XI

Fertilized Eggs

Oxygen in ccm. thiosulphate; nitrogen in ccm. $n/10$-NH_3

Number	Time	Oxygen in 180 ccm. Water		Calculated Decrease of Oxygen	Oxygen Combined by 28 mg. *N* per Hour
		Before the Experiment	After the Experiment		
34.9	1. 60 min.	15.9	11.0	7.0	4.0
	2. 60 min.	15.6	10.4	7.4	4.2
16.4	1. 60 min.	15.8	13.3	3.6	4.4
	2. 60 min.	15.6	12.8	4.0	4.9
	3. 60 min.	15.6	12.5	4.4	5.3
31.4	1. 60 min.	15.9	11.5	6.3	4.0
	2. 60 min.	15.9	10.6	7.6	4.8
	3. 60 min.	15.9	10.2	8.1	5.2
20.2	1. 60 min.	15.8	12.9	4.1	4.1
	2. 60 min.	15.8	12.8	4.3	4.3
	3. 60 min.	15.8	12.2	5.0	5.0
23.8	1. 60 min.	15.8	11.8	5.7	4.8
	2. 60 min.	15.8	11.4	6.3	5.3

fertilization the oxygen consumption had increased six or seven times.

Warburg confirmed Loeb's results that there is an increase in oxygen consumption when unfertilized eggs are treated with a hypertonic solution. In solutions of three strengths of NaCl, Warburg found a graded increase up to nine times the amount absorbed. He pointed out that this increase is not proportional to the rise in the osmotic pressure of the solution. He also found that a hypertonic solution affected the eggs so that when brought

back into sea water there is a slight increase in oxygen absorption. The more recent work on oxidation processes by Meyerhof ('11), and especially that by Shearer ('22) and by Rogers and Cole ('25) have given further and very significant information bearing on these processes in the sea-urchin's egg. Shearer shows that there is a sudden and very great inrush of oxygen within one minute after fertilization which was overlooked by the earlier workers. This increase is about eighty times that observed in the same eggs one minute before fertilization. The output of CO_2, after this first change, closely follows the oxygen consumption. Rogers and Cole recorded a great rise in temperature immediately on fertilization. It amounted to from ten to twelve times that of unfertilized eggs. It then decreases until only sixty-five per cent of that at the time of fertilization, and then remains about constant until the first cleavage. At the first cleavage it drops about ten per cent and remains constant. The different rates of development at different temperatures was utilized by Karl Peter as early as 1905 to determine whether they are of the order of magnitude of chemical reactions. He found a rather close agreement between theory and fact not only for the cleavage stages but for later stages as well.

Warburg ('08) reported from results on Arbacia pustulosa that the rate of development "gives the temperature coefficient of a chemical reaction." Thus:

28 mg. nitrogen (per hour) used at 20°C 0.7 ccm. thiosulphate
28 mg. nitrogen 28 1.4
Cal. increase for 10° = 0.9 ccm. thiosulphate

Some experiments were carried out by Loeb and Wasteneys ('11) with Arbacia to compare the rates of oxidation and the rates of development at different temperatures (Table XII). The results, they thought, show that in the ranges within which the eggs normally develop, namely between 15 and 30 degrees, the two coefficients were nearly the same. But below 15 degrees a greater divergence occurs. The temperature coefficient of oxidation does not change, but that of development increases more and more.

Fauré-Fremiet ('13) calculated the rate of the first division of the eggs of Ascaris and of Sabellaria is a function of temperature (Tables XIII-XIV). These data give a temperature coefficient that is "essentially variable" between 0 degrees and 40

TABLE XII

Temperature-Interval	Temperature Coefficient for 10°	
	Speed of Oxidation	Speed of Development
3 to 13°	2.18	
5 to 15°	2.16	
7 to 17°	2.0	
8 to 18°	.....	6
9 to 19°	.....	$\geqq 4$
10 to 20°	2.17	3.9
12 to 22°	.....	3.3
13 to 23°	2.45	3.3
15 to 25°	2.24	2.6
17 to 27°	2.0	
$17\frac{1}{2}$ to $27\frac{1}{2}$°	.....	2.2
20 to 30°	1.96	1.7
22 to 32°	1.4	
25* to 35°	1.0	

*Extrapolated value.

TABLE XIII

ASCARIS

$Q_{10} = -4.1$	between 37°	and 42°
0.0	32°	37°
+1.84	23°	32°
+3.93	16°	23°
+6.25	0°	16°

degrees. At low temperatures Q_{10} is greater than 3 and even greater than 6 in Ascaris. Fauré-Fremiet concludes that factors other than chemical ones have also an equal value in the rate of division, and he suggests from other work that he has done on Ascaris that one of these factors is the change of viscosity which his results show varies only between 5 and 5.8. "This high value, relatively constant, may account for the retardation of the mechanical phenomenon of division at low temperatures."

It should be pointed out in connection with the preceding experiments that the emphasis on the lack of constancy of the

TABLE XIV

SABELLARIA

Q_{10} =	between	and
0.0	27°	29°
−3.2	27°	27°
−1.5	23°	26°
−1.5	22°	23°
+1.27	19.5°	22°
+1.28	16.5°	19.5°
+1.26	13°	16.5°
+1.66	7°	13°
0.0	5°	7°

Q_{10} is irrelevant, because even simple chemical reactions do not possess constant values of Q_{10} (Snyder '11). The emphasis is to be placed on the order of magnitude of the coefficient and not on its invariability (Hecht '19).

Crozier ('24) has shown, by an entirely different treatment of the data of Loeb and Wasteneys ('11), that the temperature relationships, given by the Arrhenius' equation, are strikingly different in the two cases. For the relationship between temperature and oxidation rates the temperature coefficients are $\mu = 16{,}000$ and 11,800, whereas for development $\mu = 21{,}000$, and 41,000. Crozier suggests that the data of Loeb and Wasteneys, for oxygen consumption in Arbacia eggs reveal two critical points, at 14 degrees C. and 25 degrees C.[2]

Warburg ('08, '10), as stated above, found that artificial membrane formation increases the rate of oxidation of the egg as much as does the entrance of the spermatozoön. Loeb and Wasteneys ('13) also carried out experiments to test whether hypertonic solutions alter the rate of oxidation after artificial membrane formation. The rate, after artificial membranes had been formed, was determined first in normal sea water and later in hypertonic solutions.

The eggs were first treated with butyric acid, and, when they had formed membranes, one lot was put into sea water, the other

[2] The important paper of Boris Ephrussi on the temperature coefficients of the different phases of mitosis of the eggs of the sea-urchin and of Ascaris (Protoplasma I. 1926) was received too late to be reviewed in the text. The review of the oxidation—reduction potential of protoplasm by Needham and Needham, was also received too late for insertion (Protoplasma I. 1926).

into hypertonic sea water (50 cc. sea water + 8 cc. $\frac{5\,M}{2}$ NaCl). It was found that the hypertonic solution did not increase the rate of oxidation. In other words membrane formation raises the rate of oxidation to the same height as fertilization which is not increased by subsequent treatment with hypertonic solutions. This shows that the so-called curative treatment of a hypertonic solution after artificial membrane formation is not due to an increase in the rate of oxidation in the egg.

Loeb and Wasteneys could not confirm Warburg's results (on another species) that hypertonic solutions increase the rate of oxidation of eggs that have been already fertilized by sperm. They did, however, confirm Warburg's conclusion that such solutions do increase the rate of oxidation of unfertilized eggs. They explain the different effects on the two kinds of eggs as due to the formation of a membrane in the unfertilized eggs by the hypertonic solution.

The egg of the starfish is immature when taken from the ovary, i.e., the large germinal vesicle is still present. In sea water it quickly ripens and gives off its polar bodies. Loeb ('02) has shown that the ripening does not take place unless oxygen is present in the water. If after the polar bodies are given off, the egg is fertilized, there is no increase in the amount of oxygen absorbed. Loeb suggests that the difference between the behavior of the egg of the sea-urchin and that of the starfish is due to the previous absorption of enough oxygen in the starfish egg during the maturation to carry it on through the division stages.

Experiments carried out by R. S. Lillie show that the fertilized eggs of Arbacia will take up more water by osmosis than unfertilized eggs. If a mixture of unfertilized eggs and fertilized eggs is placed in sea water diluted by fresh water (40 parts sea water plus 60 parts tap water) the fertilized eggs can be distinguished from the unfertilized eggs in two or three minutes by their increased size. This difference was shown to be due to the relative resistance of the membrane to the entrance of water. The amount absorbed in the end is the same in both, but the rate is different.

In the reverse experiment, by treating eggs with hypertonic sea water, Lillie has shown that the fertilized egg loses water much more rapidly than the unfertilized egg. On the other hand starfish eggs show little difference between fertilized and unfer-

tilized eggs both in respect to exosmosis and to endosmosis. This result appears to be parallel to the oxygen consumption in the two kinds of eggs. It is not clear what this difference means.[3]

Besides these changes there are many others which will be considered when the general physiology of the whole developmental process is taken into account.

It is known that very weak solutions of potassium cyanide do not injure eggs if exposed for short periods, and from the general action of this drug on living matter it is safe to infer that its chief action is to suppress oxidations. Lyon ('02) placed eggs and embryos of sea-urchins in weak solutions of KCN in sea water and removed them at stated times to normal sea water in order to find out whether the eggs in different stages of development were injuriously affected by the KCN. If they were, the result might safely be ascribed to the need of oxygen, and it seems a fair inference that this serves us as a measure of the amount normally consumed during these periods. In general, he found a loss of resistance to KCN during the course of development. Differences were also found even for the interval between fertilization and the first cleavage. The resistance diminishes from ten to fifteen minutes after fertilization. From this time to the time of the first cleavage there is an increase in resistance. Soon after this division there is another susceptible period followed by an increase in resistance, and a similar relation was found for the interval between the second and third cleavages.

By driving out the oxygen from sea water by hydrogen gas, a comparable series of results was obtained. There was found a most susceptible period about ten minutes after fertilization, followed by a more resistant period. These results may probably be interpreted to mean that more oxidation takes place at the time when the eggs in the solution are most susceptible to its absence. There is, however, no direct evidence to prove that at the time of greater susceptibility the agents employed might not themselves produce other effects.

[3] The possible effect of anaesthetics in decreasing the permeability of the cell has been examined by Heilbrunn ('20), who points out that the evidence in support of the theory is "somewhat scanty." R. S. Lillie ('16) found that shrinkage of the egg in hypertonic solutions is delayed by the presence of ether. Heilbrunn thinks that this need not be due to a change in its permeability to water, for, in hypotonic solutions they expand just as readily in the presence of ether as in its absence.

In several papers R. S. Lillie ('09, '10, '11, '12, '14) has emphasized the importance of the change in permeability that takes place when fertilization occurs—both by the sperm and by activating agents. He has shown that the same agents that initiate artificial fertilization may cause some of the pigment of the egg to be set free, which indicates a change in permeability of the membrane (and perhaps other changes also). Lyon had already shown ('10) that a slight loss of pigment takes place in normal fertilization. At one time Lillie ('11) suggested that the unfertilized egg cannot develop because of the accumulation of carbon dioxide in it, whose escape is prevented by the surface layer of the egg. When the surface layer is changed, either by the entrance of a spermatozoön by artificial agents, the CO_2 escapes and development begins. Loeb ('16) points out that this view is hardly consistent with the fact that when an unfertilized egg is cut in two it does not develop, although the cut-surface should offer a chance for the CO_2 present in the egg to escape. He also points out that his own experiments and those of Godlewski have shown that the cortical layer of the unfertilized egg is very permeable to CO_2, hence CO_2 will cause membrane formation if present in sea water in sufficiently high concentration.

The suggestion has been made at various times that the fertilization of the egg may be concerned with the introduction of an enzyme into the egg by a spermatozoön or with the release of enzymes already present. The increasing interest in the rôle of enzymes in organic processes makes this question one of great importance at the present time. Several years ago H. Winkler ('00) made some experiments with extracts of spermatozoa of sea-urchins. The extracts, he supposed, caused the eggs of some species to begin to segment. Gies ('01) repeated the experiment more carefully with negative results. It is not improbable that other changes were introduced by Winkler that brought about the results. Cremer also obtained only negative results with extracts of fish sperm. Loeb (Dynamics '06) reported some experiments that might have been expected to indicate that the development is initiated by the activation of a catalyst, and concluded that the idea that the spermatozoön carries a positive catalyst into the egg had, at that time, received no support.

A series of qualitative measurements of the amount of two oxidizing ferments, namely peroxidase and a catalase present in

the ovary and testis of amphibians were made by Wolfgang Ostwald ('07), who reported relatively different amounts in the two organs in question. Sperm extracts he found contained, in equivalent concentrations, three times as much catalase as the egg extract. Likewise the sperm extract contains more peroxidase than the egg extract. Mixtures of the two with respect to both oxidases were tested. Immediately after mixing there was no change, but in one case examined there was an increase of 23 per cent after standing for four hours. It is doubtful whether this result can be interpreted in relation to fertilization. Ostwald has discussed at length the rôle of ferments in development, contrasting his earlier "coagulation theory" with Loeb's "chemical theory." He argues that the different agents used to induce artificial fertilization also initiate auto-oxidations. These lead to chemical syntheses of nuclein substance which bring about orienting and localizing precipitations (astrospheres). This conclusion recalls other suggestions that have been made as to the rôle of astropheres in artificial parthenogenesis (Wilson, E. B. Delage, Herlant, Chambers, etc.). It may appear very questionable whether it is possible to bring into line the reported difference in amount of enzymes in egg- and sperm-extract with the normal fertilization when it is recalled that the size of the spermatozoön is insignificant compared with that of the egg.

Lyon and Terry ('07) reported that their earlier tests for a catalase in the eggs of Arbacia seemed to show that there is more present before than after fertilization, but later Lyon ('09) became doubtful of the value of this method, and repeated the experiments. The new results indicated that when entire eggs of the sea-urchin are treated with hydrogen peroxide, more oxygen is set free by fertilized eggs than by unfertilized. The change begins about three minutes after fertilization and reaches a maximum at about 20 minutes. No further increase was demonstrated. The result may mean that the sperm carries in a kinase or some activating body, but it may mean only that the results are due to the known change in permeability of the egg-membrane, so that the peroxide and the enzyme come more easily together. No decision between these alternatives was reached.[3]

[3] Stehle and McCarty ('20) have found that there may be great variations in the rate of animal metabolism without any corresponding change in the catalase content of the blood; and Seymour ('20) has pointed out that there is no satisfactory method for a quantitative determination of catalase in solid tissues.

CHAPTER X

CLEAVAGE AND THE MECHANISM OF CLEAVAGE

The cleavage of eggs offers by far the best opportunity for the experimental study of cell-division, because eggs are free cells that can often be procured in immense numbers, all at the same stage and due to divide at a definite time after fertilization at a given temperature.

The division into two equal parts is generally regarded as the typical division (Fig. 21*a*), and while many eggs do divide at first into two equal halves (or blastomeres) other kinds of eggs divide unequally. In many eggs the first division is complete before the second begins, but in other eggs the first division may not have passed completely through the egg before preparations for the second begin. In some eggs the constriction starts as a ring around the egg, but in most eggs of this type the constriction begins a little sooner at the pole, and passes more rapidly from the pole inwards than it does from other parts of the periphery. In a few eggs, as in the ctenophores (Fig. 132), the constriction beginning at the polar field cuts through from that point inward, finally completing itself at the opposite pole. There are other eggs, rather large as a rule, and containing much yolk, in which the segmentation nucleus divides into two, but no superficial cytoplasmic division occurs at the time. Many insects and crustaceans show this type of cleavage (Fig. 22*a*, *b*). Further nuclear divisions take place as the nuclei gradually move to the surface layer of the egg (Fig. 22*c*). After the nuclei have reached the surface the superficial layer of protoplasm divides, each nucleus becoming the center of a cell.

When an egg cleaves, its blastomeres tend to round up at each division into a sphere. This is, as a rule, interfered with by the confining membrane which keeps the blastomeres pressed against each other (Fig. 21*a*), but even when the membrane is removed, or is not present immediately around the egg, the

blastomeres, after rounding up, still come together and flatten one against the other. As a result of their coming together they assume a definite pattern that is characteristic for each type of egg. Embryologists have studied in great detail the arrangement of the blastomeres in the cleavage stages. The patterns shown at each stage are so constant that in many cases the indi-

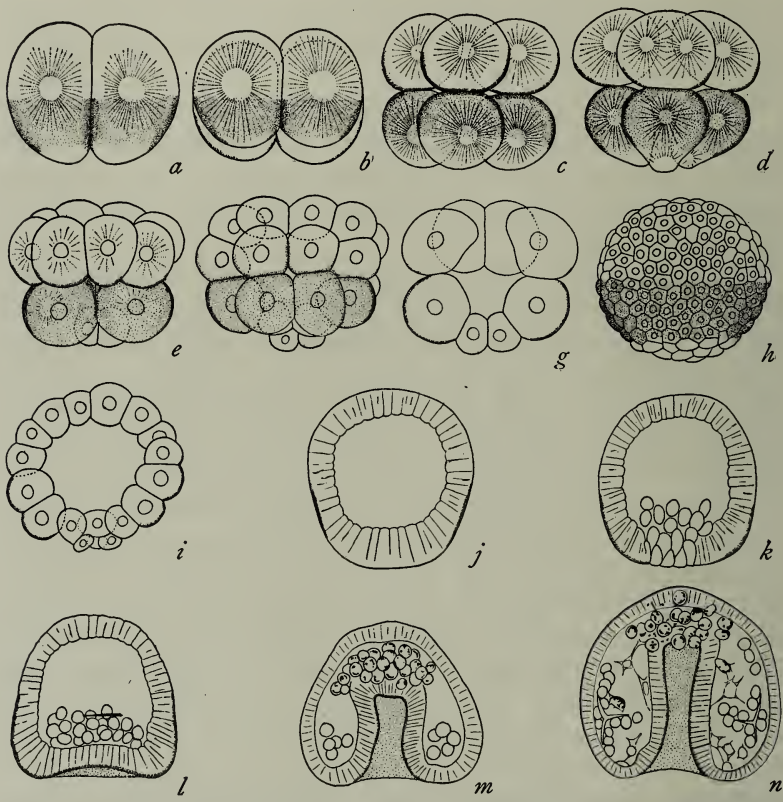


FIG. 21.—Cleavage and gastrulation of the egg of Paracentrotus. (After Boveri

vidual cells can be traced to the embryonic organs that develop from them. Studies of these relations have been particularly successful where the blastomeres are few in number at the time when the embryonic organs can be identified.

Some of the same patterns are present in widely different groups that have very different end stages. The simplest pattern, in a descriptive sense, is found when an egg becomes divided successively in three planes at right angles to each other (eight-cell

stage) as seen both in the small, sea-urchin egg (Fig. 21*a*, *b*, *c*) and in the relatively large egg of the frog (Fig. 23). At the fourth division of the sea-urchin egg four small cells, the micromeres, are cut off around the antipole, while the four cells of the opposite hemisphere divide meridionally into equal cells (Fig. 21*e*). In the frog, the four somewhat smaller black cells (of the eight-

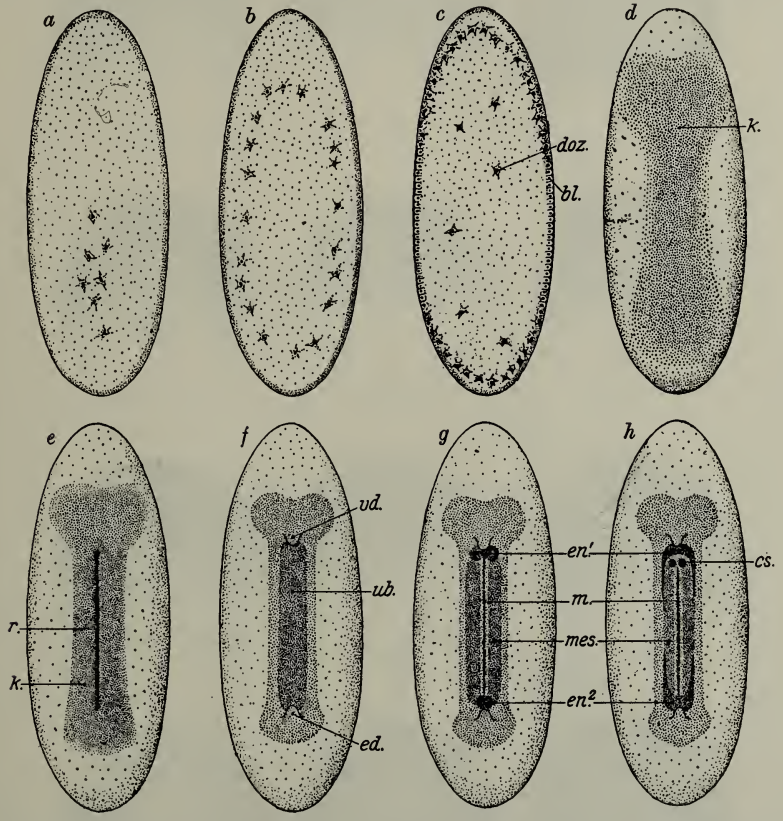


Fig. 22.—Cleavage and formation of the embryo of an insect. (After Mangold.)

cell stage) divide equally, but in different planes in different cells, while the four, somewhat larger "yolk cells" divide unequally (Fig. 23*e*).

An interesting and much studied type, called the spiral type, is shown by the eggs of many molluscs, annelids, nemertines and of some planarians (Fig. 24*A*, *B*). The first division is often into unequal parts, but in other cases is equal (Fig. 37*a*, *b*). In

the former type the smaller cell divides into equal parts, the larger into unequal parts of which the smaller is approximately the same size as each of the two smaller products of the division of the

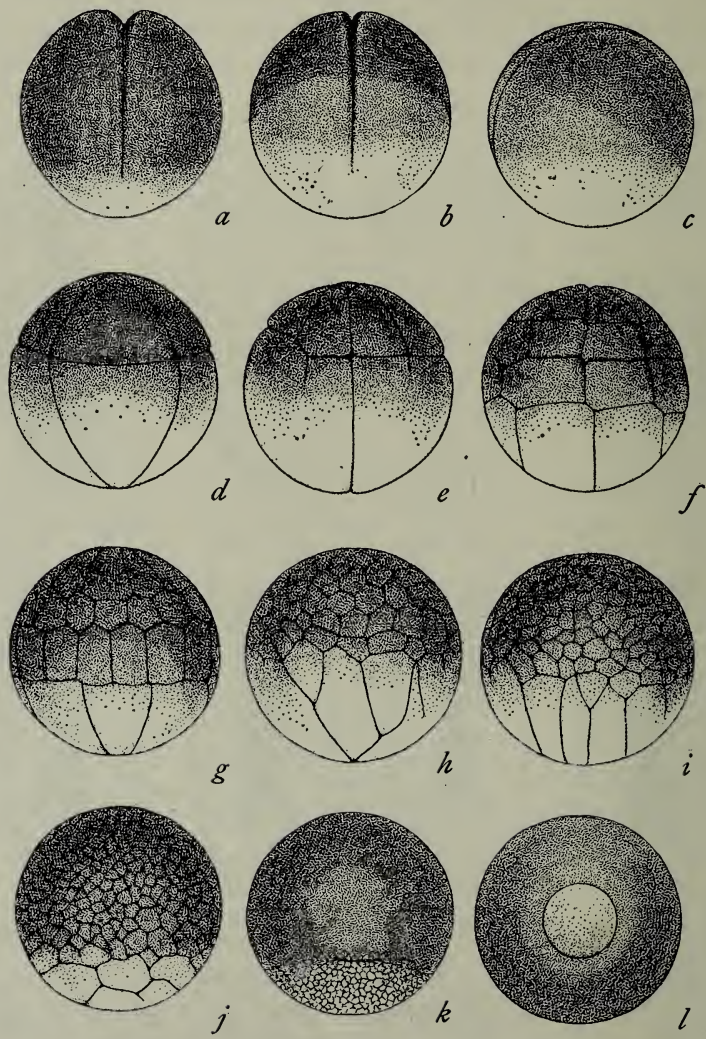


FIG. 23.—Cleavage of frog's egg. (After Morgan.)

sister cell (Fig. 24*B*). These four cells may be described as lying in a plane around the pole, but, in fact, two of the four often meet across the pole to form a cross-furrow, which serves

as a convenient landmark. It lies, as seen in polar view, characteristically in a right- or left-handed direction with respect to the earlier divisions. At the third division (Fig. 24*C*), each of the four cells produces a small cell from its polar end, a micromere. Moreover each micromere does not lie in a meridional position with respect to its sister blastomere, but to the right (or to the left) of the meridian of that blastomere. If the micromere

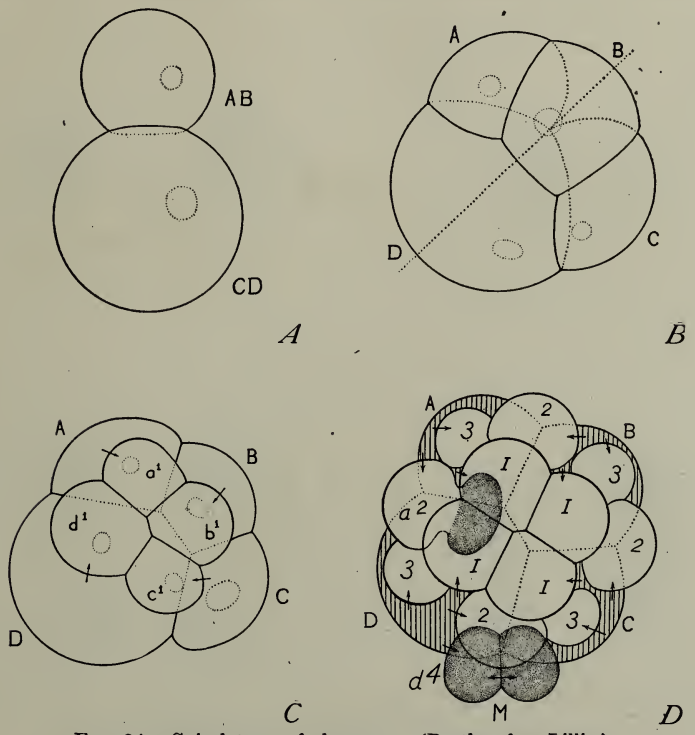


Fig. 24.—Spiral type of cleavage. (Partly after Lillie.)

lies to the right, as seen from above the pole, the cleavage is said to be dextral or in the direction of the hands of a watch (clockwise); if the micromere lies to the left, the cleavage is sinistral. Each of these types is specific, often for large classes, but sometimes, even within the same species, some individuals may produce sinistral, others dextral types of cleavage.

There is said to be a bilateral type of cleavage, but this refers generally either to the future plane of bilateral symmetry of the embryo, as in the frog, or to the position of the cleavage plane

with respect to the bilateral form of the egg, as in the squid. There are, however, a few eggs, such as those of the ascidian (Fig. 25) and Amphioxus (Fig. 113), which, after the second division at least, have a bilateral pattern. Here the first division is into equal cells, the second division is slightly unequal, and two larger or posterior cells and two smaller or anterior cells are

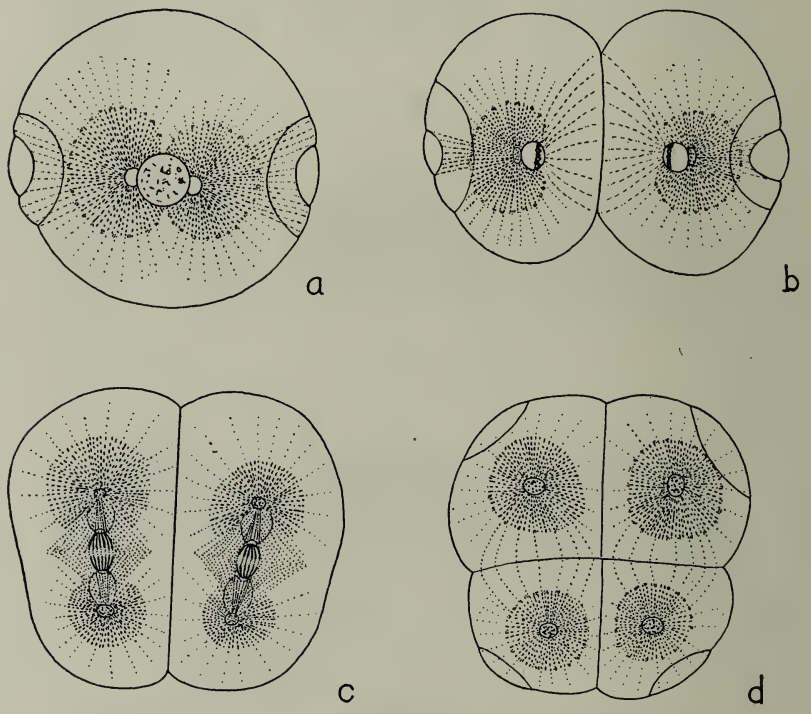


Fig. 25.—Cleavage of ascidian egg. (After Van Beneden.)

present.[1] The plane of the first cleavage then becomes a plane of bilateral symmetry of the egg and also of the late embryo.

Many variations of these cleavage patterns are familiar to embryologists, and other types are also known, but those briefly described above are the ones most frequently met with in experimental work.

[1] This is true also in most cases in the frog's egg, in relation to the gray crescent and is also seen later in the relative sizes of the cells, on opposite sides of the blastula. (Fig. 23 h and i.)

Protoplasmic Movements of the Materials of the Egg Prior to Cleavage

Extensive movements of the materials of the egg are known to take place in some eggs just prior to the first division. In other eggs, where such movements have not been described, it is not improbable that similar, if less extensive changes, take place. These changes appear to be extremely important, not only because they are supposed to be related to the type of cleavage that takes place, but also because the redistribution may have an intimate relation to the later differentiation of the different blastomeres. Few questions in embryology are more debatable at present than the interpretation of the significance of the distribution of the materials of the egg as the cause of its later differentiation. It is a fact worth noting, in passing, that in cases where extreme movements of the cytoplasm take place there has been up to that time no predetermined plane of cleavage; while conversely, in other eggs where no such readjustment has been reported, the plane of symmetry is already determined by the shape of the egg itself, which may mean no more than the shape imposed on it by the enveloping membranes.

The point of extrusion of the polar bodies as well as the entrance point of the sperm appear to be centers of movement, and it is probable in several of these cases that the penetration path of the sperm is directly or indirectly connected with the first appearance of bilaterality in the egg.

The breaking down or absorption of the nuclear wall and the setting free in the protoplasm of the more fluid nuclear sap are generally regarded as inaugurating the cytoplasmic movements. The nuclear sap mixes with the surrounding protoplasm, the chromosome threads condense into chromosomes surrounded by the mixture of sap and cytoplasm; a spindle develops with the chromosomes at its equator; and while these changes take place (which may occur in a few minutes) there begins a flow of material toward the pole of the egg. The spindle is carried in the stream, and is turned, as it moves, into a radial position. One of its ends comes to the pole, where a protrusion of protoplasm occurs that includes little more than the outer end of the spindle (which is now much reduced in size). The protrusion is pinched off, and a very unequal cell-division has taken place.

These changes that lead to the formation of the first polar body are, in some eggs at least, accompanied by movements throughout the whole egg—in fact they could scarcely take place without some rearrangements; and the movements are at times so extensive that they cannot fail to give the impression that they have a deeper significance, and have, as a matter of fact, been so interpreted. But it is quite possible that their significance may have been greatly exaggerated. The movements may be no more than a streaming that accompanies the migration of the polar spindle to the surface of the egg. Since the first polar spindle is often formed before the egg is fertilized, the movements that accompany its migration, or are the cause of its change of position, cannot be supposed to have any necessary connection with fertilization, but rather with the peculiar type of cell-division taking place in the formation of the polar-body when a minute, almost microscopic cell is one partner and the egg the other. In fact, the disparity in size is so great that it is customary to speak of the egg as giving off, or extruding, its polar body.

A few examples will serve to illustrate the changes that take place, but it is to be remembered that these are probably extreme examples; for, in many eggs it is supposed that the alterations are more local and do not involve such extensive rearrangements.

The maturation of the egg of Chaetopterus has been described by F. R. Lillie ('06). The ovarian egg (Fig. 26*a*) has a large nucleus that comprises about one-eighth the volume of the egg. The egg is attached to the ovarian wall at its antipole (vegetative pole). There is an outer ectosarc over two-thirds of the polar surface, while at the attachment pole (antipole) this layer is lacking, or at least less developed, and the endoplasm comes nearer to the surface. At the free end there is a thinning of the ectosarc, and the endoplasm comes quite to the surface. This polar field is not cut through in Fig. 26*a*, but is shown in Fig. 26*b*. Yolk granules are present in the polar hemisphere, which also contains more of the watery (?) vacuoles. The ectoplasm contains large colorless spherules, which have been shown to stain in Orange G.

When the egg-nucleus breaks down, and the interior begins to flow to the pole, the ectosarc moves toward, and comes to cover the "vegetative" hemisphere (Fig. 26*b*). At the same time it retracts to some extent from the polar field. The endoplasm

moves outward around the egg (under the ectosarc), and in part into the vegetative hemisphere. It may seem probable that this shift, as well as that of the ectosarc, is part of the same general movement. The final changes are seen in Fig. 26*c*, where the first polar body has been given off, and the spindle for the second division is present, as well as the sperm-asters.

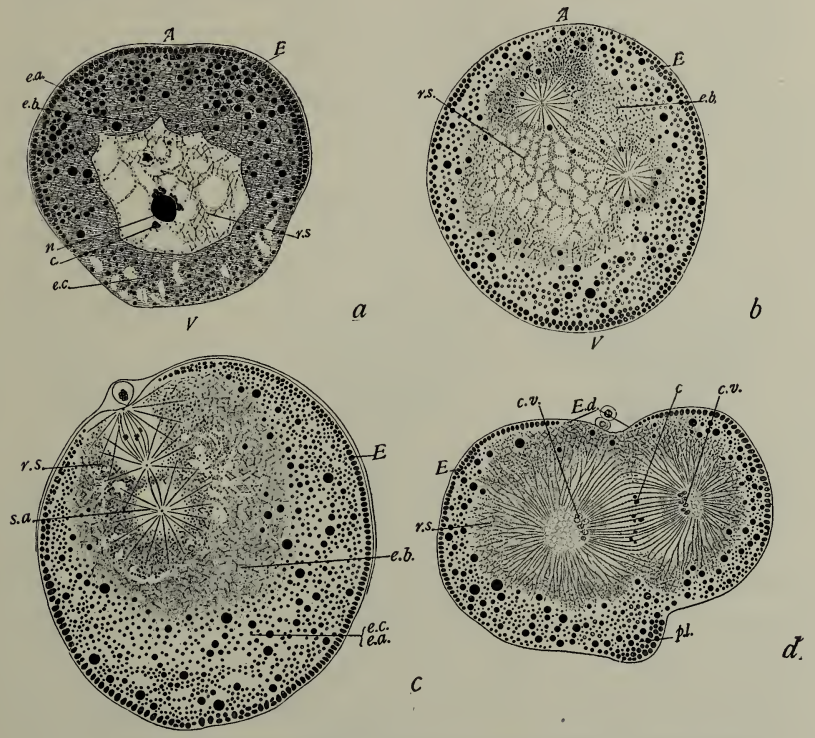


FIG. 26.—Maturation stages of the egg of Chaetopterus. (After Lillie.)

The extensive cytoplasmic movements that take place in the living eggs of some of the fresh-water snails (Physa, Lymnaea, Planorbis) have been described by Conklin ('10). The clear area, that appears in the interior when the egg-nucleus dissolves, moves toward the pole until the end of the spindle reaches that point. The clear area then spreads out like a cap around the pole. The first polar body is formed at the pole after two hours, and the second polar body one hour later. At the moment of extrusion of both polar bodies, the outline of the egg becomes irregular.

The clear cap next spreads over the upper hemisphere. The sperm-nucleus approaches the egg-nucleus. They meet beneath the animal pole. Under them lies a finely granular "yellow substance" in which later the segmentation spindle develops. The first cleavage-constriction appears, and, as it cuts through the egg, the clear protoplasm surrounds both blastomeres. These observations show that extensive rearrangements take place in the molluscan egg during the maturation and cleavage stages.

The egg of the sea-urchin (Fig. 27*a*) gives off its two polar bodies while in the ovary. The egg-pronucleus comes to lie excentrically without respect to the primary axis, and, so far as known, without respect to the future plane of cleavage. Boveri ('01) has shown for the egg of Strongylocentrotus lividus that

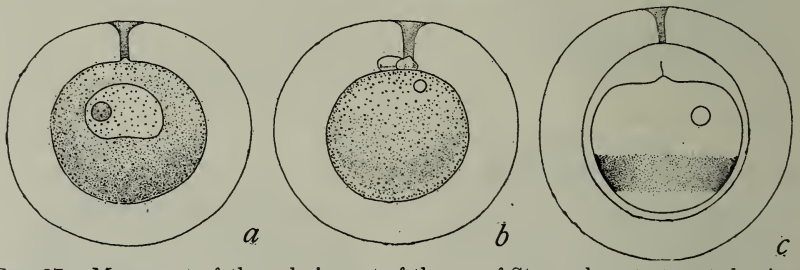


Fig. 27.—Movement of the red pigment of the egg of Strongylocentrotus at the time of fertilization. (After Boveri.)

when the spermatozoön enters the egg, the scattered red pigment-granules move, or are carried toward the equator where they form a ring lying for the most part below the equator, i.e., in the antipolar hemisphere (Fig. 27*c*). There is no evidence that the ring has at this time a bilateral form. The two pronuclei come together in the primary axis of the egg, somewhat on the polar side of the center. The first cleavage-plane, which cuts through the pole and the primary axis, lies at right angles to the ring as seen in Fig. 21*a*. In other species of sea-urchins, e.g., Arbacia, there is no evidence of movement of pigment granules as in Strongylocentrotus until the micromeres are formed, when the granules at the pole are pushed aside as the spindle comes to the surface.

The protoplasmic movements that take place in the egg of the ascidian, Styela (Cynthia) partita, have been carefully followed by Conklin ('05). The egg, when laid, has already formed

its first polar spindle, and is waiting in the resting condition for the entrance of the spermatozoön to inaugurate a new set of changes. The egg contains many scattered, yellow granules,

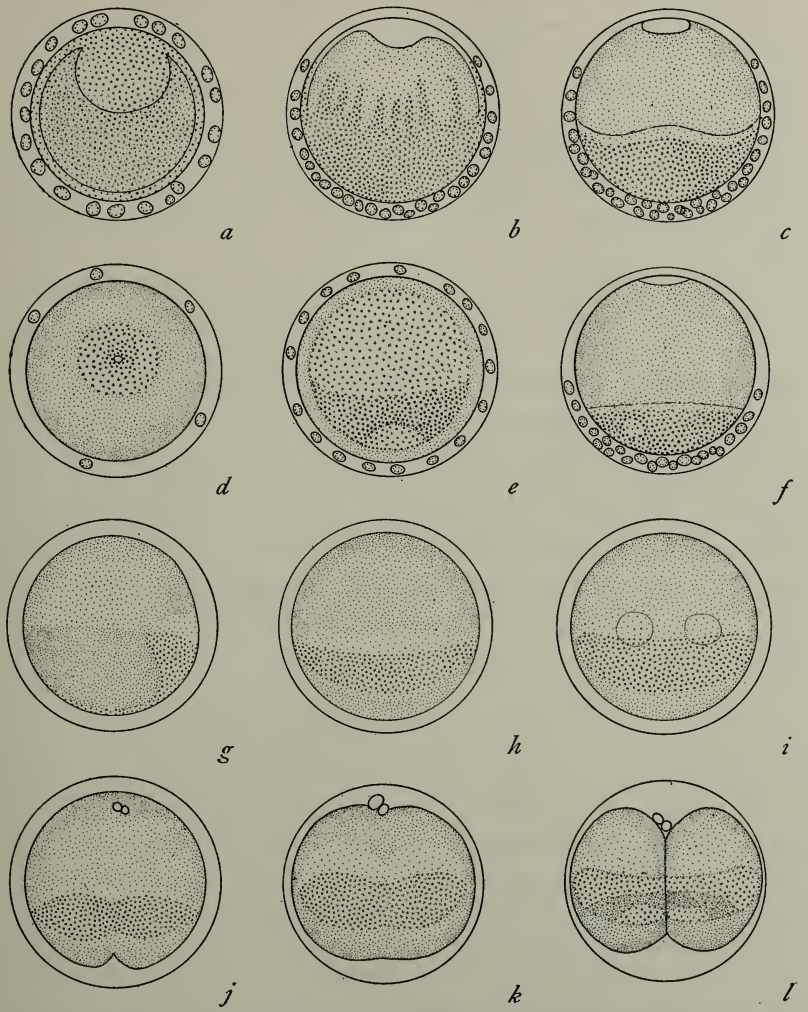


Fig. 28.—Movements of the materials of the egg of the ascidian, Styela, at the time of fertilization. (After Conklin.)

especially in the more superficial layers (Fig. 28*a*). The sperm enters at or near the antipole, and the first polar body is given off, followed by the second one. The sperm-head and its accom-

panying aster now begin to move inward, and the egg-pronucleus also moves inward, both taking a course to one side of the axis. They finally meet excentrically. In the meantime the yellow granules have begun to shift toward the antipole (Fig. 28*b-f*). The ring is thickened on one side, and finally assumes the form of a crescent whose thickest part indicates the plane in which the first division takes place (Fig. 28*g*, *h*, *i*). The movement of the yellow granules serves as an index of the extensive cytoplasmic changes through which the granules are carried along. These rearrangements are intimately bound up with the formation of the future median axis of the embryo, and will be described later.

How far these protoplasmic movements, following fertilization, are merely incidental to the change taking place in the egg during maturation and fertilization is perhaps open to question. It is quite possible, of course, that while only incidental at this time, they may, nevertheless, be essential for the later progress of development; or while some of these changes may have little or no significance, others may be important. Furthermore, the observed movements and alteration of position of the materials of the egg are probably only the visible effects of more profound changes that cannot be seen. The subsequent movements of material that take place during cleavage will be described in other connections.

The Mechanism of Cleavage

In the cleavage of the egg, all of the features characteristic of cell-division are present. Since the time of cleavage can be regulated through insemination, and since the cleavages follow each other rapidly and at definite intervals, the egg has been extensively used for the study of division. Yet, despite the large number of studies of cleavage that have been made, the physical aspects of cell-division are not understood. Most of the work has been concerned with a description of the cleavage-pattern as seen on the surface of the living egg, which was supplemented by a study of the interior of the cell as seen in dead and preserved and stained material. These patterns and preparations have given rise to a number of suggestions as to the nature of cell-division and, since the time of the division of the cell is closely correlated

with changes in the mitotic figure, most of the discussion by cytologists has revolved about this internal phenomenon.

It should be observed, however, that the mitotic figures, on which these speculations rest, are in nearly all cases those seen in preserved material. How far these appearances are reliable indices of the configuration of the living materials of the cell, or egg, is by no means settled. Comparisons between the stages of living and dead material leave little doubt but that, in the main features at least, the preserved (coagulated) mitotic figure gives, in a general way, a picture of the arrangements of the materials present in the living cell. When, however, the question arises as to the physical and chemical nature of the changes that take place when the polar asters and the central spindle of the mitotic figure develop in the living cell, the evidence from preserved (dead) materials can be used only with much caution.

A different line of attack on the mechanism of cleavage has been made by those biologists who have sought to show by models how certain physical principles might be appealed to, to explain the division of a fluid sphere. Other biologists have produced *simulacra* of the mitotic figure by diffusion currents in albumen, or in other semi-fluid media. And still others have drawn attention to lines of force in a magnetic field that resemble the lines seen in a preserved spindle. There is, in addition, some information gained in the course of experimental work on the living material that bears on the problem of cleavage. While all these sources of information must be taken into account, I shall refer here only briefly to the interpretations based on the appearance of the preserved mitotic figure, and deal more at length with the experimental evidence derived from the living egg.

The attachment of the fibres of the central spindle to the chromosomes, at the time when the daughter halves move away from each other, suggested that the fibres might be contractile, and pull the chromosomes towards the poles of the spindle. If not contractile, the fibres might still be more solid lines attaching the chromosomes to the poles, and as the poles move apart they would drag the chromosomes with them (Klein '78, Van Beneden '83 and '87, Boveri '88, Heidenhain '95).

The resemblance of the coagulated mitotic figure to the lines of force in a magnetic field (Fol '73, Ziegler '95, Gallardo '96–09, Hartog '05, and others), has led to several attempts to explain

the changes taking place during mitosis as an electrical phenomenon. Ralph Lillie's ('03) work, that went to show that the two poles have the same electric signs, and are alike osmotically, shows that the mitotic and magnetic fields are essentially different. Prenant ('10) and Hartog ('05, '14) have postulated, therefore, that the mitotic figure is the seat of a special kind of force, etc. Resemblances between the mitotic field and a field of gelatin in which osmotic strains have been induced have also been considered.

The study of floating, suspended, and also of sunken drops of oil, that can be made to divide by causing in them a difference in surface tension at the poles and at the equator, has been much more suggestive, not only because division of the drop can be brought about in this way, but also because the currents set up at the time of division follow the same course as the currents that have often been described when eggs or cells divide. Bütschli ('00) suggested (following Quincke '90) that the constriction of the cell is the result of movements accompanying surface tension—a band of greater surface tension developing in the region of the cleavage furrow. Erlanger ('97) had recorded the surface movements of granules in the egg of nematodes towards the plane of cleavage, and this observation has been later confirmed by Spek and others. Gardiner ('95) had still earlier made similar observations on the egg of a planarian. Several similar observations have later been recorded by others. That movements take place cannot be doubted, but how they are related to the division is not so clear.

Robertson ('09) floated a drop of olive oil on water and laid across it a string moistened with soap. The drop divided into two. Since soap decreases the surface tension between oil and water, he concluded that the division was due to the lowered surface tension in the plane of division. McClendon ('11) pointed out that the situation was more involved than assumed by Robertson, and suggested that the pull on the string caused the division of the drop in Robertson's experiment. McClendon submerged the drop of oil by adding enough alcohol to the solution to cause the drop to sink below the surface. When he brought soap-solution in a pipette, or a solid piece of soap, near one side of the drop it bulged towards the soap, indicating that an outflow and not a constriction takes place where the surface tension is lower.

Later, McClendon ('12), showed that if two pipettes containing a solution of NaOH are brought near to a submerged drop of oil at opposite points (poles), the drop constricts in an equatorial plane between the poles where the surface tension is greater than at the poles. At the poles the surface tension is lowered by the NaOH solutions.

Robertson showed later that even submerged drops might also be divided by a soap-string laid across the drop, meeting apparently McClendon's criticism of his earlier experiment; but McClendon points out that the result is here due to the weight of the string that sinks into the egg where the surface tension of the drop is lowered by the alkali on the string.

Spek ('18, '20) has still later carried out numerous experiments with different oil drops and has studied their divisions when two crystals of NaOH are brought close to the surface at opposite poles. If the oil has the right consistency (rape seed oil + olive oil + chloroform) the drop will be cut in two at the equator where surface tension is highest. If small bubbles of air or pigment granules are mixed with the oil, currents can be seen in it. An internal, axial stream moves outward towards the points where the crystals are near the surface and currents move from these two points over the surface of the drop towards the equator (Fig. 29) where division of the drop may take place. These, and other similar experiments led Spek to support McClendon's view that division of the drop takes place where the surface tension is highest. Spek's observations on dividing nematode eggs (Fig. 30) also show that interior and superficial currents, like those seen in the drop of oil, are present during division.

Gray ('24) has used a model in which drops of dilute alcohol surrounded by a covering of oil are suspended in dilute alcohol. The system is a two-phase system comparable in this respect to a system of soap bubbles that is also a two-phase system, but the oil-alcohol model gives a pattern more like that of the blastomeres, since the angles formed at the contact-faces of air-bubbles are not quite the same as those in the two-cell stage of the egg. A drop of olive oil is immersed in a mixture of alcohol and water that has the same specific gravity as the oil. By means of a fine capillary pipette two drops of the same alcohol mixture are injected into the drop of oil (Fig. 31*a*). Some of the oil may then be gradually removed. The interior droplets assume the

forms shown in Figs. 31*b* to *e*. The form of the system depends on the tension exerted by the outer surface of the oil, and on the resistence of the two drops of alcohol to deformation by

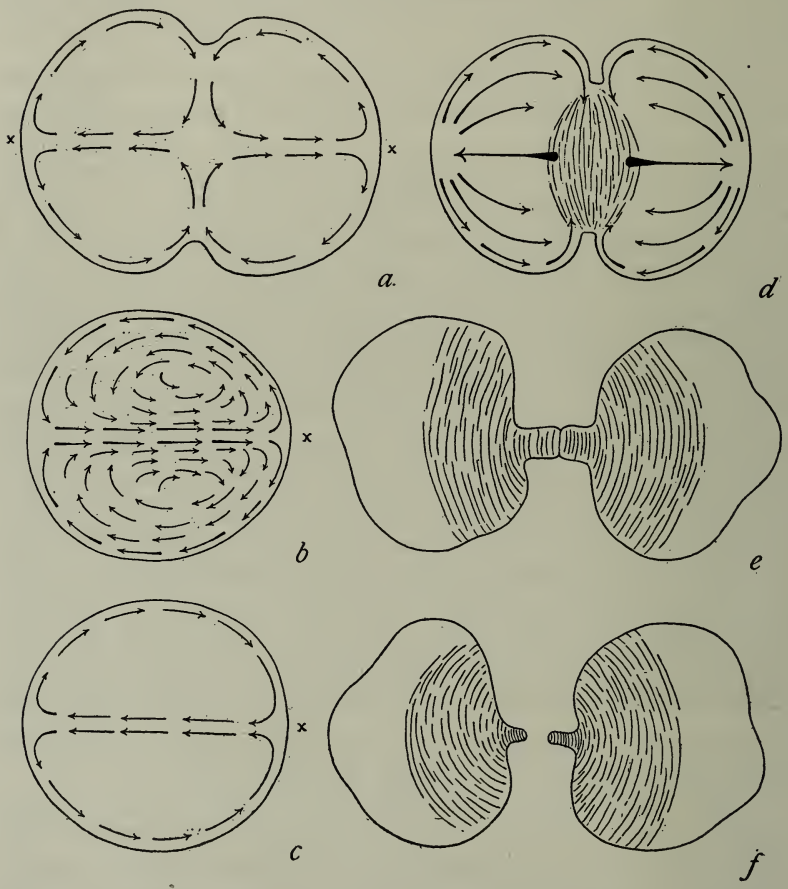


Fig. 29. *a*, theoretical streaming in an oil drop whose surface tension is lowered at opposite sides (x–x); *b*, streaming in an oil drop whose surface tension is lowered at one side (x); *c*, streaming in an oil drop whose surface tension is raised at one side (x); *d*, *e*, *f*, cutting in two of a drop of oil when two crystals of soda arebrought near to opposite poles. (After Spek.)

pressure. These forces are "probably" both simply the expression of the outer facial tension between the oil and the alcohol.[2] These

[2] An analogous system could be developed by means of solids if they were elastic. (Gray.)

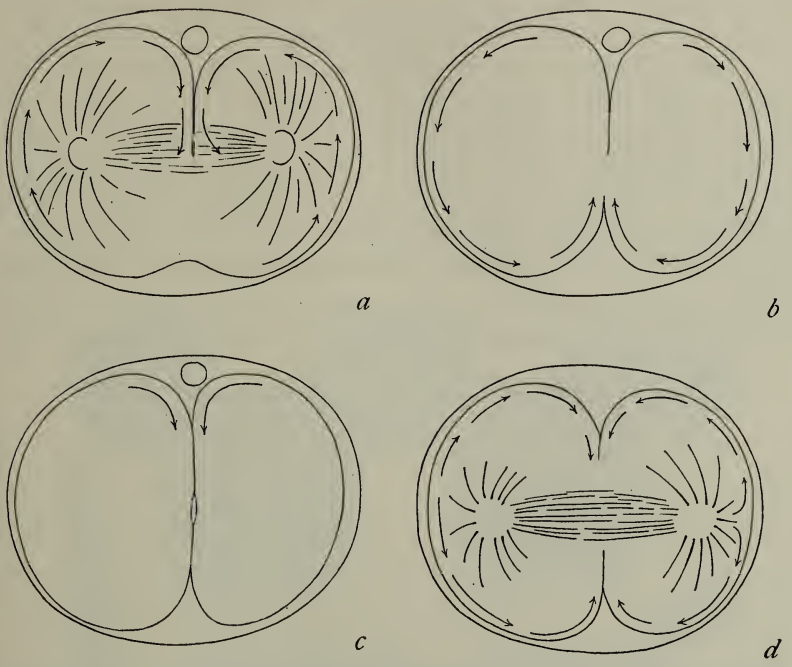


FIG. 30.—*a*, *b*, *c*, cleavage of egg of Rhabditis dolichura, showing reversals of streaming during cleavage; *d*, same egg constricting from all sides at once showing streams in opposite directions. (After Spek.)

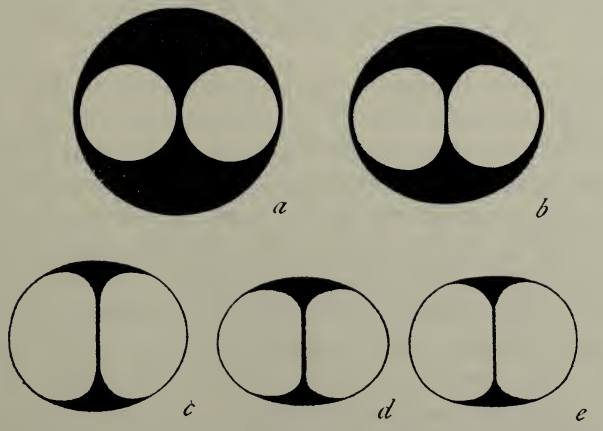


FIG. 31.—*a*, two drops of water in a drop of oil. As the oil is withdrawn the water drops assume the form shown in *b*, *c*, *d*, *e*. (After Gray.)

compressed drops bear a close resemblance to the forms assumed by the two blastomeres of the divided egg.

Gray's study of the formation of the ectosarc of the sea-urchin's egg (the hyaloplasma membrane of other writers), and his experimental studies on the effect of the medium on the shape of the blastomeres have led him to conclusions concerning the factors in cell-division that are different in several respects from those appealed to by other observers. Gray finds that about a quarter of an hour after the fertilization membrane of the egg of Echinus has been thrown off, a marked change begins to occur at the surface of the egg (Fig. 32). Up to this time it has been

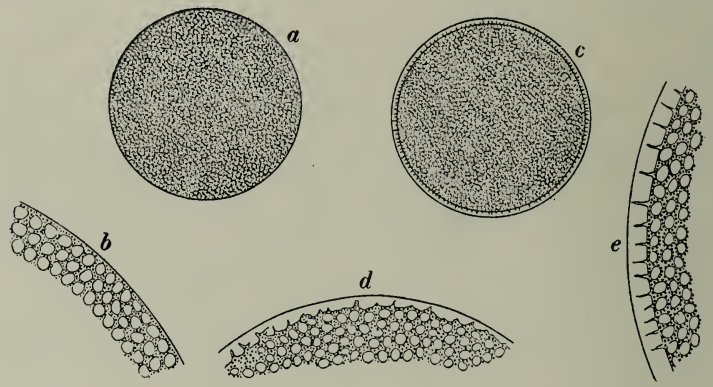


FIG. 32.—Unfertilized or newly fertilized egg of Echinus: *a*, with the fertilization membrane omitted; *b*, surface of fertilized or newly fertilized egg enlarged; *c*, fertilized egg, thirty minutes after fertilization; *d*, surface of fertilized egg enlarged; *e*, surface of fertilized egg when formation of ectoplasm is nearly complete. (After Gray.)

smooth, but now begins to develop a series of fine irregular transparent processes free from microsomes. These processes gradually fuse together to form a clear layer of ectoplasm over the surface of the egg.[3] The ectoplasm is complete half-an-hour after fertilization (Fig. 32*c*, *d*, *e*). Just before the first cleavage, when the egg slightly elongates, the ectoplasm begins to thicken in the plane of the future division (Fig. 33*b*) and thins out at the ends of the egg. As the cleavage furrow deepens, the ectoplasm in the furrow becomes more marked (Fig. 33*c*, *d*, *e*), and follows into the crevice between the two halves. When the division is complete each blastomere is surrounded by a layer of ectoplasm

[3] First described by E. A. Andrews ('97) for the sea-urchin; similar membranes were noted by Hammar ('96).

(Fig. 33*f*, *g*). While the bulk of the ectoplasm is thus distributing itself, at the equator of the fully divided egg, as if it were a fluid immiscible with the endoplasm, the outer surface of the ectoplasm shows very well-marked wrinkles, indicating its solid nature. The form of the fully divided egg is due to the properties of the ectoplasm. The proof of this is found by placing the fully divided egg in a calcium-free sea water. The cells separate from each other, as Herbst ('00) first showed, and become spherical (Fig. 35*c*). An examination of the eggs in calcium-free sea water

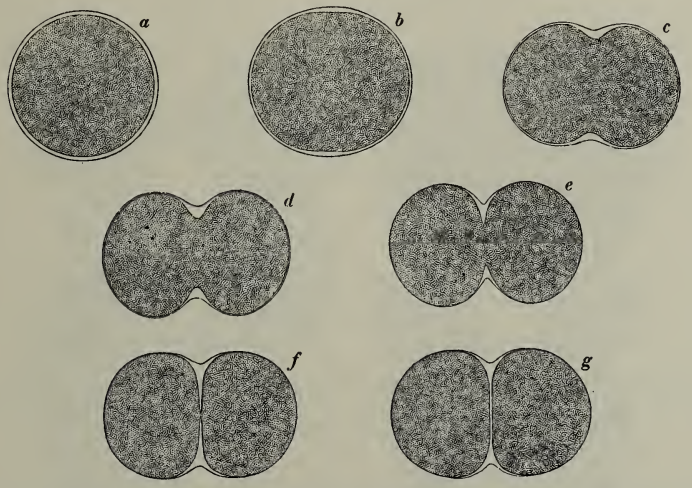


FIG. 33.—Normal cleavage of the egg of Echinus, showing how the endoplasm becomes completely separated into two parts surrounded by ectoplasm. (After Gray.)

shows that the outer surface of the ectoplasm disappears. Some of it remains for a time between the two blastomeres because it is the last to dissolve. If a fertilized egg is placed at once in calcium-free sea water, no definite ectoplasm is formed, and the development of the egg stops. If a living egg is cut across by a fine needle, the cytoplasm on the cut surface consists of a sticky mass, but if cut in calcium-free sea water the endoplasm flows out in a stream, and is mixed with the sea water. This leads to the conclusion that the outer surface of the ectoplasm is normally due to the effect of calcium of the sea water on the substance exuding from the surface when the ectoplasm is formed. (Gray.)

If unfertilized eggs are put into hypertonic sea water (50 cc. sea water + 10 cc. 2½ Mol. Van't Hoff's solution), the whole egg

shrinks, often irregularly. On the other hand if fertilized eggs with an ectoplasmic layer are placed in the same solution they suffer only a slight reduction in size; the endoplasm especially shrinks, but the ectoplasmic layer becomes wider than is normally the case. Gray interprets this result to mean that the ectoplasm absorbs water, and increases in width while the endoplasm loses water.

If, after the first division, the eggs are transferred to hypertonic sea water, the ectoplasm swells at once, and the two endo-

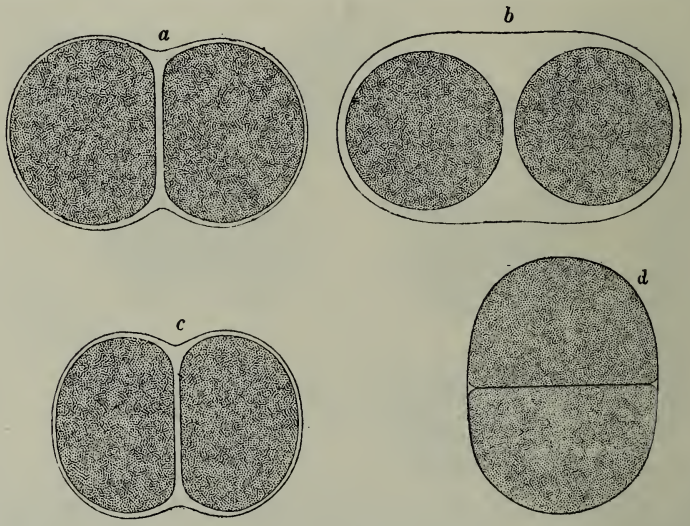


FIG. 34.—In the first figure, *a*, the cleavage has just been completed in sea water; *b*, the egg was then transferred to hypertonic sea water where the ectoplasm swells and the endoplasm contracts to two spherical masses; *c* and *d*, the same egg in acid hypertonic sea water. (After Gray.)

plasmic masses become spherical (Fig. 34*b*). The effect is the same as when some of the alcohol is removed from the two droplets, when the droplets become more spherical.

If acid is now added to the hypertonic solution the ectoplasm contracts owing to loss of water, and resumes its pressure on the enclosed masses of endoplasm which begin to flatten against each other (Fig. 35*a*, *b*). That the result is due to the ectoplasmic surface is shown by the effect of an acid solution on eggs that have cleaved in calcium-free sea water (Fig. 35*c*, *d*). The deformation includes only these regions where the ectoplasm is still present.

Most cytologists are agreed that the presence of two asters in the egg about to divide is intimately connected with the cleavage, although, as has been stated, different views have been suggested as to their function. Gray has recently advanced certain evidence to show that the force exerted by the growing asters (which tends to elongate the egg in one direction) "causes such a redistribution of ectoplasm that division results." This evidence is as follows:

That the force that tends to elongate the egg before cleavage is opposed by the pressure of the ectoplasm can be shown by suppressing the ectoplasm by means of calcium-free sea water during the actual process of cleavage. Its pressure is thereby diminished, consequently the endoplasm is freer to elongate and in each half to become spherical as shown in Fig. 35*c*.

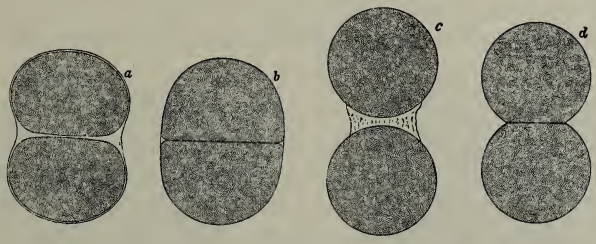


FIG. 35.—*a*, normal egg in sea water; *b*, the same egg in acid sea water; *c*, egg with ectoplasm removed by Ca-free sea water; *d*, same egg in Ca-free sea water plus acid. (After Gray.)

As shown by Wilson ('01) the asters in eggs can be made to disappear by placing them in sea water to which a little ether has been added. The cleavage process comes to a standstill. If the eggs are then returned to pure sea water, the asters reappear and division occurs, or else they may divide to form two new asters, which may then act as centers of further division. Unless the asters reach the surface of the egg no division takes place. Gray placed eggs in a 2.5 per cent solution of ether in sea water, then, after a short time, transferred them to sea water containing only 0.05 per cent of ether. The asters reformed, but remained small. Nuclear division took place, but the nuclei remained close together, and no cleavage occurred. The process may repeat itself until the whole egg is filled with asters. In some eggs these come to the surface, when furrows appear between the nuclei during a division period. This evidence shows that the size as well as the position of the asters is closely associated with

the appearance of cleavage furrows, as Boveri and others had first shown. Gray's conclusions may be summed up as follows: The two asters of the dividing egg enlarge until practically all of the endoplasm is absorbed by them (or changed from a sol to a gel condition). The diameter of each aster then approaches half that of the whole egg as seen in Fig. 36*a*, *b*. As the asters enlarge they push apart and cause an elongation of the egg. As a result in the change in shape, the ectoplasm from the sides flows toward the equator where it becomes thicker and passes into the cleavage furrow between the two masses of endoplasm until finally each endoplasmic sphere is surrounded by ectoplasm. In fact, the cleavage which separates the endoplasm has left the halves united by a common ectoplasm. When the asters fade out the ectoplasm in each half-sphere can reassert its power, and bring the two endoplasmic spheres together into a spherical form again,

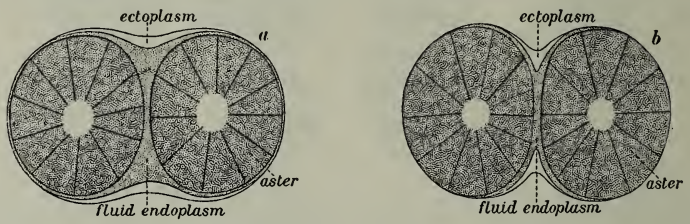


FIG. 36.—Diagram to show the redistribution of the cell phases caused by enlarging asters. (After Gray.)

but they do not fuse together, since a layer of ectoplasm lies between them.

While it cannot be doubted that the ectoplasm changes its appearance, as Gray describes, it may be possible that some of the change in amount of ectoplasm at different points during cleavage may be due to a local increase or decrease in the ectoplasm, rather than due to a flow or change in position. It seems probable, nevertheless, that to some extent an actual movement does take place as described. It is not entirely clear, if the ectoplasm is a "solid" or semi-solid, how it would flow so freely from point to point unless it, too, may change at times its consistency, but it is dangerous to make such assumptions to suit each special situation unless otherwise proven; for, such assumptions give the impression of playing fast-and-loose with the hypothesis of a semi-solid membrane. The separation of the endoplasm into two complete hemispheres, in Gray's account, seems to imply something

more than the simple opposition of the asters as they enlarge. Whatever influence is present, that causes the solidification of the endoplasm around the asters as centers, would seem to involve other and perhaps potent factors in the end result; for, if each half of the endoplasm should concentrate into a perfect sphere (except so far as prevented by the egg membrane or by the ectoplasm) as implied in the diagram (Fig. 36), the ectosarc, if a fluid, would automatically move into the region between them and come to surround each in a complete layer without necessarily its presence or redistribution being the immediate cause of the result.

That the presence of an outer somewhat stiffer layer around the fertilized egg plays a significant rôle in cleavage seems, however, well established by numerous observers. Admitting this, makes it difficult to apply McClendon's explanation of surface tension as the causal agent in the development of such eggs. That a common external sticky layer is present around each blastomere enclosing a more fluid interior is now generally admitted and that the common envelope is an element both in determining the shape of the individual blastomere, as well as the shape of the combined blastomeres (that gives the characteristic pattern of cleaving eggs), can scarcely be doubted in the light of the evidence now available. In addition to this, it is also probable that there are conditions of the endoplasm, such as the change from a single to a two-fold system, or a change from one physical state to another (sol to gel), that antagonize the surface forces of the ectoplasm and may ultimately bring about new arrangements.

Gray has pointed out, in connection with the observations of McClendon's and Robertson's as applied to living cells, that they assume the surface of the blastomeres to be liquid. This, he thinks, is open to question on the ground of his studies of the ectoplasm of the sea-urchin's egg. Gray also points out that McClendon and Robertson leave their analogy at the point where the cleavage is just completed while the most striking feature of the fully divided cell is that the two blastomeres show no tendency to fuse with each other. Oil drops readily unite, unless prevented by a protecting layer on the surface of the oil sufficiently strong to oppose the operation of surface forces. "There is no evidence that either McClendon's or Robertson's experiments would succeed under such conditions."

The more direct experimental evidence concerning cell-division relates to a change of physical state taking place in an egg about to divide. Certain parts of the egg pass from a more fluid (sol) to a more solid (gel) stage. The evidence for this comes from several sources. A few observers had noticed that the sea-urchin's egg, centrifuged before fertilization, is stratified by a smaller centrifugal force (or in a shorter time) than after fertilization. Heilbrunn ('15, '20, '26) has utilized this fact in a carefully controlled series of experiments, and has shown that the change gradually comes on as the egg prepares to divide. Eggs from a single female were separated into two lots; one of the lots was fertilized. Ten minutes later one lot was put into one tube of a hand centrifuge, and the other lot into the other tube. They were revolved for 18 seconds at 180.5 revolutions per second. The eggs were immediately examined. The unfertilized lot was typically stratified. None of the fertilized eggs showed any trace of stratification. Even two and a half minutes after insemination, no effect on the fertilized eggs could be shown. In other experiments it has been found that, before the second division, the blastomeres are again more easily stratified. The stratification is due to the movements of the granules and of droplets suspended in the protoplasm according to their specific gravity. Their rate of movement will depend on the fluidity of the medium.

Heilbrunn ('20) has also shown by the same method that those reagents that initiate "artificial fertilization" (which he points out consists for the most part in starting cell-division) also cause a stiffening of the protoplasm. For instance, unfertilized eggs were placed in NaCl hypertonic sea water, and left there for 25 minutes.. They were then tested against unfertilized eggs in sea water by revolving both sets for 19 seconds at the rate of 171 revolutions per second. The normal eggs were found to be completely stratified, while the NaCl eggs showed no stratification.

Similar tests of eggs subjected to other agents, known to incite cleavage, gave the same kind of results. Even such substances as toluol and saponin were shown to cause gelatinization or coagulation of the egg-cytoplasm.

In another way Heilbrunn demonstrated that after fertilization, and also after the application of those agents that incite parthenogenesis, there takes place a hardening of the cytoplasm.

It was first determined at what height a small piece of glass must be dropped on to an unfertilized egg in order to crush it. If the same piece of glass is dropped from the same height on a fertilized egg (deprived of its membrane) it resists the impact. When the unfertilized egg bursts, its contents pour out into the water, but the contents of a fertilized egg, if it be made to burst, exudes more slowly, showing that a physical change takes place in the egg about to segment that represents a change from sol to gel. Heilbrunn concludes that the initiation of development, either artificially induced, or as the result of fertilization, always involves a gelatinization or coagulation of the egg. This coagulative change begins before the egg interior shows any other signs of the approach of cleavage. The egg substance (or substances) is evidently easily coagulated. Only a slight alteration in the salt concentration of the medium is then sufficient to start such a change. The fact that both increase and decrease of salt-concentration are effective, suggests that the protein involved is of the globulin type. The mitotic spindle probably arises as a direct result of the coagulative change. The actual explanation as to how this occurs is a problem of colloid chemistry. It has been shown that coagulation of proteins can produce structures similar in certain aspects to the mitotic spindle.

By a somewhat different procedure it has been shown that the spheres at the poles of the mitotic figure, and in fact the entire figure, represents a more solid material surrounded by a more fluid medium. If at the time when the mitotic figure is well developed the eggs are placed on a centrifuge, the mitotic figure may rotate as a whole, and come to lie parallel to the direction of the force, the more stable position for the figure. It is moved through the egg and under ordinary circumstances is not separated into its component parts. The chromosomes are, as it were, frozen in the center of the semi-solid material.

The movements of the asters in polyspermic frogs' eggs described by Brachet and by Herlant become explicable on the view that the growing sperm-asters are more solid spheres floating in a more fluid medium. As they grow they push each other apart, and come to occupy positions in respect to each other that semi-solid bodies would assume in a more fluid sphere (Figs. 7, 8).

It is interesting to compare, from this point of view, the position of the cleavage-planes in compressed eggs, since their position

is determined by the location of the asters. As the asters enlarge in an egg under compression and tend to push away from each other they will move into the most stable position, which would seem to be parallel to the compressing plates because there is more unoccupied space parallel to the plates. No doubt other conditions in the cell also operate at the same time. The final position of the asters is probably determined by more than one factor. Perhaps more significant are the changes that take place in an egg placed under compression at the time when the bipolar mitotic figure is well developed. Unfortunately the results are not entirely simple, for, it appears that as the pressure is applied to the eggs, they tend to slip around so that the figure is often brought parallel to the plates. Moreover the egg itself may have already begun to elongate when the mitotic figure has reached a considerable size, and the position that the egg takes between the plates is partly determined by its shape. But there appear to be occasional cases when the egg is caught and compressed in the direction of the spindle or oblique to it. The mitotic figure then moves within the egg until it is parallel to the plates.

The egg-cutting experiments are also of interest in this connection. If the egg is cut before the polar asters have much enlarged, their size will be determined by the amount of material left in the piece from which they must obtain their material in order to "grow." Hence they are reduced in size, and it seems reasonable to explain the proportionate form of the cleavage that results, by the relative sizes of the asters. Such an interpretation removes at least the mystery with which this phenomenon of proportionate cleavage has been surrounded.

Experimental evidence relating to the physical condition of the eggs comes also from the exploratory needle of the micro-dissection instrument as worked out by Chambers.[4]

Chambers ('17, '19) has studied by micro-dissection the different phases of the egg in process of division, more especially in the transparent egg of the sand dollar, Echinarachnius.

A very fine glass needle bent sharply at the end is used in dissecting the egg. It is held in a Barber instrument or holder that allows the needle to be directed at will by the experimenter. The egg lies in a drop of water suspended from the under side of a cover-slip that lies over a moist chamber. When the mitotic

[4] See also account in Chapter IX.

figure of the dividing egg of Echinarachnius has reached its fullest development there is present at each pole a large aster. The center of the aster in the living egg contains a clear fluid sphere from which radiate an enormous number of narrow rays which appear to be made up of the same fluid substance as the central sphere. The rays are broadest near the central sphere and taper along their course, losing their identity before reaching the surface. The granular cytoplasm lies around (between) the more fluid rays and may be said to have a radial structure; in fact, these may be the so-called rays (fibres) of coagulated (dead and stained) material. If the end of the needle is pushed into the central sphere it can be moved about without disturbing the surroundings, indicating that the center is in the sol stage. When the nucleus lies in the sphere it may be pushed about there with ease. On the other hand, the substance between the fluid rays (the cone-like projections of cytoplasm) are more solid in comparison (gels). These parts may be bent or pulled about by the needle and act as comparatively rigid gelatinous structures with the cytoplasmic granules immovably imbedded in them. The entire ring (or sphere) through which the fluid rays extend is solid in comparison with the more liquid cytoplasm of the resting egg. "In short, dissection of a cell containing a fully developed aster gives one the impression that the cytoplasm is in the gel stage, while the sphere and its rays are liquid."

The gel state is most pronounced in the region nearest the central sphere, diminishing towards the periphery. Owing to the comparative stability of the gel, the aster may be much distorted without being destroyed, it may even be twisted by the needle into a spiral shape. When the needle is removed the aster may (or may not) return to its original form. Extensive tearing of the aster, however, may bring about the loss of the radial appearance, the entire cell reversing from the gel to the sol state. It can be made to disappear by churning the cytoplasm with the needle, but even then the fluid may again collect in a sphere and the rays may reappear.

The development of the mitotic figure was also followed, and its structure in different stages was micro-dissected. Two or three minutes after the entrance of the sperm a tiny aster appears that rapidly enlarges. From the beginning it contains a liquid center. Around it the cytoplasm has already begun to harden

for the aster at this stage may be pushed or rolled in the surrounding liquid cytoplasm. The sperm-nucleus is firmly held in the gel, and is dragged about by the aster. The aster slowly moves to the center of the egg as it enlarges and carries the nucleus with it. The sperm-nucleus comes to lie in the central sphere. It may be dragged out of the sphere, but so long as it is within the confines of the aster, it will, on being released, move back into the sphere. As long as the egg-nucleus lies beyond the confines of the aster it does not move, but when the extending rays of the aster reach it, it moves into the central sphere and comes in contact there with the sperm-nucleus. Its passage into the aster may be explained by the assumption of a centripetal current between the rays. That such a current exists Chambers thinks probable from the following observation: Occasionally small oil-like droplets are found in the egg. If one of these is pushed by the needle into the periphery of the aster it will move along the rays towards the center.

After the two nuclei have met, and have begun to enlarge, the ray-like structure that forms a halo around them begins to fade out. This is due to a reversal from gel to sol, as shown by the fact that the needle, on being dragged through the cytoplasm, now carries no distorted threads in its wake, and pushes the granules about as though they were embedded in a liquid that is slightly viscous. This condition lasts but a few seconds. It seems to correspond with the formation of two asters on opposite sides of the nucleus, which supposedly involves the loss or rearrangement of the first formed, sperm-astral system, with an aster at each pole. The rays soon blaze out again, but now around two centers. The rays extend almost to the periphery of the egg, and the needle shows that the cytoplasm is again a comparatively rigid gel.

When the amphiaster is fully formed, the region between the poles in which the chromosomes now lie lengthens out into an oval body. Its bulging equator retreats until its sides are parallel. A pause of some duration then follows. In about ten minutes a change takes place in the cytoplasm about the equator, which appears to be a reversal from gel to sol, although this was not positively ascertained. An intrusion of granular material into the equatorial region of the central body takes place. Following this, a constriction appears on the surface of the egg, that seems

to be due to a liquefaction of the cytoplasm in the equatorial plane, while elsewhere it remains in the gel stage. The rays disappear from this region. By the time cell-division is completed, all of the cytoplasm has returned to the sol stage and each cell contains a single aster.

The series of changes just described show that during the division of the egg a definite series of events takes place that involves extensive changes from gel to sol in certain regions of the egg. The constriction of the egg—cell-division—takes place when two opposed masses in the gel condition are present with a sol phase between them.

It should be observed, in passing, that while it may be possible to interpret the more fluid centrosphere of each aster at its greatest development as due to the flowing into the center of new fluid materials between the "rays" of the aster, it is also not impossible that the fluid in the center may be due to the liquefaction of the inner portion of the sphere itself—a process that later overtakes the entire sphere. The evidence furnished by Chambers in favor of a flow to a center between the rays cannot be regarded as sufficient, because the rate of flow that was inferred by the measurements of occasional granules in it, would appear greater than that required for all the peripheral fluid to move to the center. Besides this *a priori* objection it may be pointed out that other physical conditions might cause the observed movements. It is generally agreed that there is present on the surface of the egg a semi-solid layer of protoplasm, sometimes called ectosarc or hyaloplasm. Whether this ever becomes entirely liquefied at any stage of cleavage seems doubtful. The superficial movements of granules, seen at cell division, may take place beneath the superficial layer without involving it in the movements. On the other hand, if the similarly directed movements in the oil drops are due to surface tension, or are indicative of changes in surface tension it seems probable that these movements involve the outer layers of the drop of oil as well as the layers inside them. It may seem doubtful, therefore, how far one is justified in identifying the two movements in question, or in other words if a hardened layer is present over the cell, whether the phenomenon of its division is comparable with that of a homogeneous oil drop.

It is not obvious whether the gelation of regions of the interior of the egg at the time of division can be brought into line with

the experiments on oil drops from which it appeared that division of the drop is due to an increase of surface tension in the plane of division. It may be argued that the approach of the asters to the surface may cause a lowering of surface tension at opposite poles leading to a relative increase of tension at the equator, but there are no apparent grounds for assuming that the approach of two spheres of gels to the surface would lower there the tension. Nevertheless, the relation between the presence of the two gelatinized spheres in the egg bears such a definite relation to the plane of cleavage that one is tempted to search for some connection between the two. If, then, as seems not improbable these two spheres of relatively more solid protoplasm fill up most of the interior of the two halves, the question arises whether when such a condition is reached the more fluid protoplasm lying around these spheres and in between them at the equator might not draw together around each half producing in consequence the observed constriction (division) at the equator. The result would depend of course on the consistency of the semi-fluid superficial shell of protoplasm over the spheres.

Physical Influences that Affect the Cleavage Pattern

When the blastomeres are closely confined by an enveloping inelastic membrane, it tends to make them, collectively, assume a spherical form, and in consequence alter the shape of the individual blastomeres. How far the pressure of the membrane influences the arrangement and the form of the cells can be studied by removing the membrane. After the removal of the membrane of the sea-urchin's egg, the first two blastomeres push further apart, than when inside the membrane, but later they come together again, flattening against each other, and together they assume the same shape as when enclosed in the membrane. The resulting shape of each blastomere is then nearly the same as when the membrane is present. If the membrane is sufficiently elastic to be stretched somewhat during the first division, its pressure might be supposed to assist the other physical factors that bring the cells together after division.

The eggs of some molluscs float freely in a fluid jelly enclosed by a membrane. After each cleavage the blastomeres flatten against each other, as in eggs having a close membrane, and the

mass as a whole assumes a nearly spherical form. It is evident, therefore, that other agents than the membrane are present that suffice to bring the blastomeres together, and determine their form.

Several physical principles have been appealed to as capable of explaining the form of the blastomeres during cleavage. For example, the configuration of the blastomeres has often been compared with that of a group of soap bubbles. Plateau ('73) first explained the physical principle that regulates the shapes of soap bubbles in contact with one another. According to laws of capillarity, two or more bubbles, if brought in contact, flatten against each other. The principle involved demands that the sum of the external surfaces shall be a minimum, or expressed in another way, that the total surface energy shall be a minimum. Two bubbles coming in contact decrease by apposition their surface energy without the disappearance of the separating film. The bubbles move freely over each other until the arrangement of their surfaces gives the minimum of surface energy. According to this principle of least surfaces, not more than three surfaces can meet in a line, and not more than four surfaces can meet in a point.

When two or more blastomeres are in contact they seem to conform to this principle, although other factors may modify their arrangement. If the blastomeres are not free, as are soap bubbles, to move over each other, their arrangement may be different.

Robert ('02) has imitated all of the early cleavage patterns of the dividing egg of the mollusc, Trochus (Fig. 37), by means of soap bubbles. The bubbles were placed in small, somewhat concave, porcelain dishes. Four bubbles of equal size (Fig. 38*a*, *d*), assume the forms shown by four blastomeres (Fig. 37*c–f*). On top of four such bubbles, four smaller ones can be placed (Fig. 38*e*), and if these come to lie alternately with the larger ones below, they imitate very closely the 8-cell stage of Trochus, including the characteristic cross-furrow (Fig. 37*h*).

According to Jenkinson ('09) a closer imitation of cell-configuration is shown by drops of albumen suspended between xylol and oil of cloves (to which a little alcohol has been added). Each such drop has a superficial membrane by which adjacent drops adhere. Because of this surface-film, the drops flatten against

each other as do soap bubbles, but since the surface-films of a drop of albumen are semi-solid, the drops are unable to move as freely over each other as are soap bubbles. They furnish therefore a better model of segmentation. If four smaller drops of

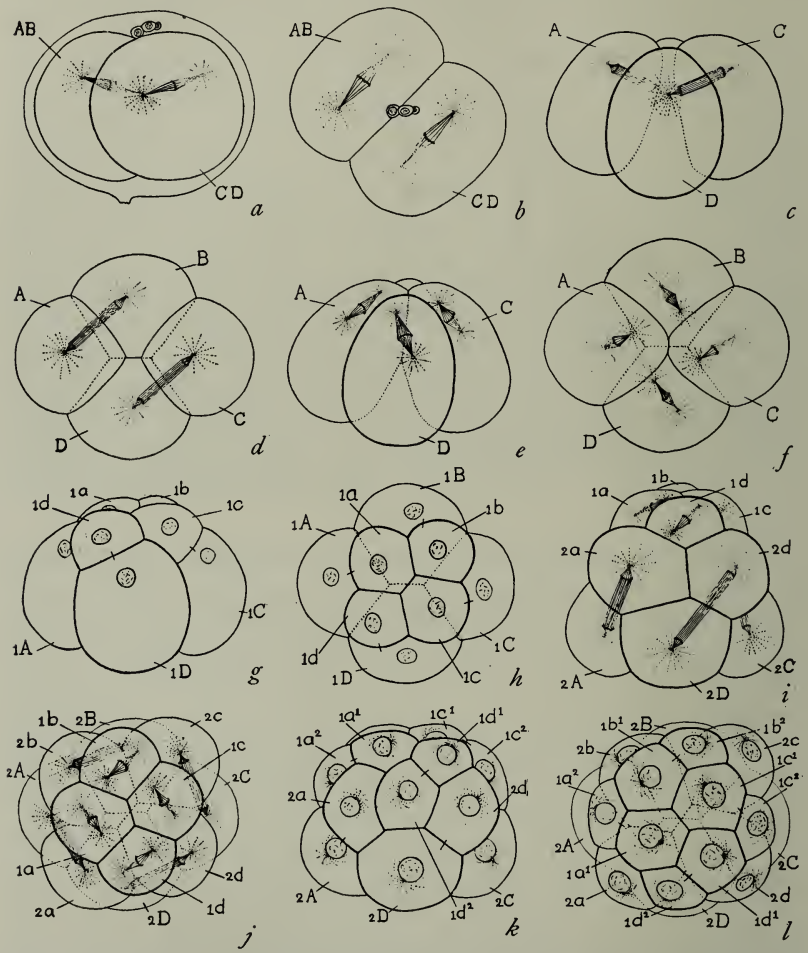


Fig. 37.—Cleavage of the egg of the mollusc, Trochus; for comparison with the arrangement of soap-bubbles shown in Fig. 38. (After Robert.)

the albumen are cut off from four larger ones the smaller ones remain superimposed, while small oil drops tend to slip in between the larger ones. The tendency of a drop of albumen to remain near the point of origin is important when comparisons are made

with living cells, for in these the position of a blastomere is to a large extent predetermined by the position of the mitotic spindle. The spindle first orients itself obliquely to the axis of the cell, bringing the four micromeres of the mollusc's egg, for example, more nearly into the interspaces between the macromeres (Fig. 37*e*, *h*). Their position is due, then, to their mode of origin

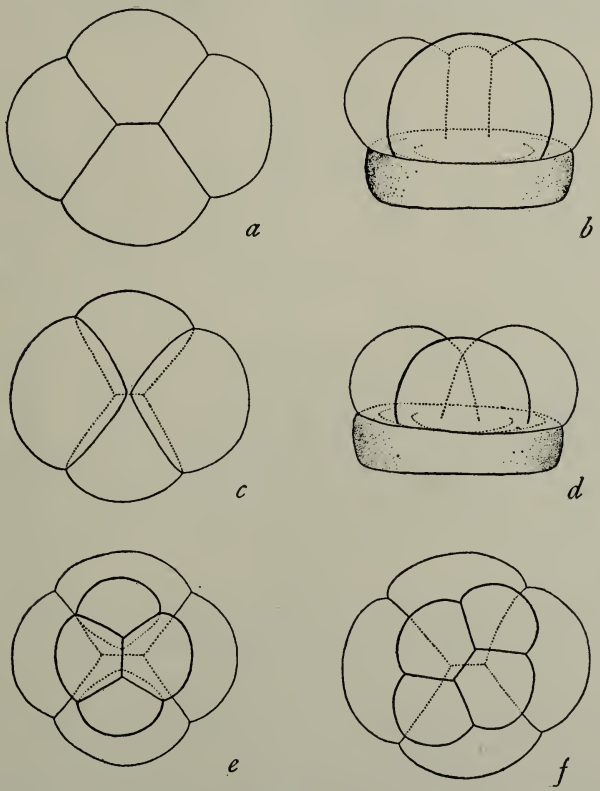


FIG. 38.—Diagram showing the arrangement of soap-bubbles in a slightly concave dish. Compare with cleavage stages of Trochus, shown in Fig. 37. (After Robert.)

rather than to rearrangements, after division, in accordance with the laws of capillarity.

Roux ('96, '97) made many experiments with drops of paraffin oil suspended between alcohol and water (or between alcohols of different grades). A little calcium acetate added to the medium helps to keep the drops from floating apart. The drops must be confined in a vessel (cylinder) a little larger than the first

drop; otherwise the droplets will move apart as they are separated. The pressure of the walls of the vessel on the periphery of the group holds the drops together. If a drop is divided into two, each half flattens against the other, and each assumes the form shown in Fig. 39*A*. If four drops of equal size are present they assume the arrangement shown in Fig. 39*B*. There is generally a small opening in the middle. If divided into four parts of unequal sizes, the four drops take the form shown in Fig. 39*D*.

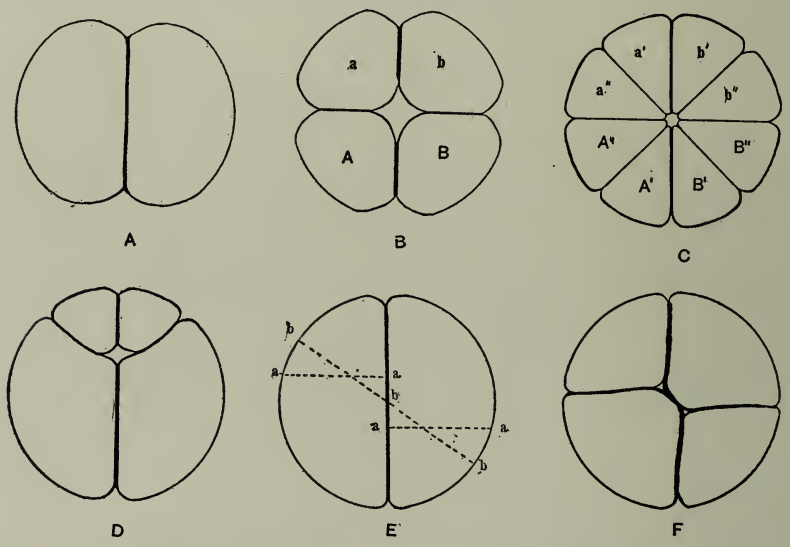


FIG. 39.—Diagrams of arrangement of oil drops floating in alcohol and confined in a glass cylinder of such a size that the oil drops are under mutual pressure. (After Roux.)

If, by division of two drops, two large and two small ones are made in such a way that they alternate (Fig. 39*E*), they assume the relative position shown in Fig. 39*F*. Here the two large drops are united in the center by a broad "cross-furrow."

Eight drops formed from four by radial division assume the arrangement shown in Fig. 39*C*. If the planes of division are not strictly radial, but are nearly parallel to the first plane of division, the resulting arrangement is that of Fig. 40*C*. Finally if four small drops are cut off at the periphery of four larger ones, they remain at the periphery, alternating with the larger drops (Fig. 40*E*).

Many other arrangements of the drops can be brought about, but these will suffice to illustrate how similar such systems are to the patterns shown by many dividing eggs.

The inability of blastomeres to shift from their place of origin is shown by removing one of them from the group. The remaining blastomeres may then change their positions, but only to a slight extent, showing that after a group is once formed the cells do not freely glide over each other as do soap bubbles or oil drops. The failure of readjustment is probably due, as Roux has pointed out, to the sticking together of the blastomeres at their surfaces of contact.

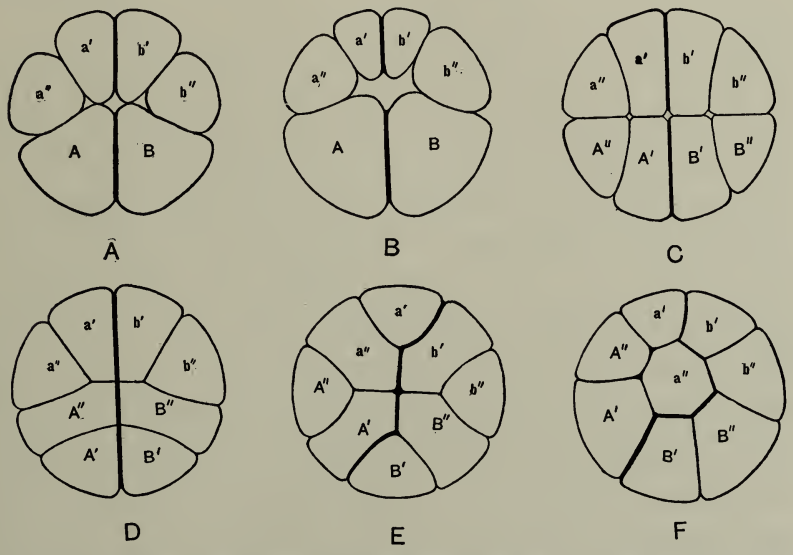


FIG. 40.—Diagram of arrangements of oil drops floating in alcohol as in Fig. 39. (After Roux.)

The work of embryologists on cell-lineage has shown that the future position of cells is frequently indicated by the position of the spindle in the parent-cell that is about to divide; and the position of the spindle itself can often be traced back to the movement of the asters that become the poles of the spindles, and even to the position of the asters at the close of the last division (Conklin). In other words, the cells are not to be thought of as first dividing in a haphazard way, and their arrangements subsequently determined by the compressing membrane, or by the laws of capillarity. On the contrary these latter physical agents appear to play a secondary rôle in the determination of the cleavage pattern. The determining factors are those that are operative in fixing the position of the spindle in the cells at suc-

cessive divisions. Conklin's work on the egg of Crepidula is especially noteworthy in tracing out the internal changes that culminate in the position assumed by the spindle before division.

The question has been raised as to whether the coming together of the blastomere, after each cleavage, may not be due to some other physical or physiological principle than those discussed above. In this connection Roux's early experiments with the isolated cells of the frog's blastula are significant. Roux teased apart cells of the early blastula in a drop of salt-solution, or, better, in a drop of dilute albumen of the hen's egg. Many of the cells become separated and sink to the bottom of the drop. In the course of a few minutes Roux observed that if two such cells are not further apart than one fifth of their diameter, they often send out protrusions towards each other, and if these protrusions meet they fuse, and the two cells are drawn together and round up into a single sphere. Even groups of several cells may unite in this way. This coming together he called cytotaxis. Cytotaxis or chemotaxis is, according to Roux, a sort of crawling movement that may be compared to the movements of an amoeba. There is nothing in this view to suggest that in cytotaxis some unknown kind of attraction draws the cells together. On the contrary, it may be due, as Roux suggests, to substances exuding from the cells in the space between them. In this space, the exudate or secretion would be twice as concentrated as elsewhere over their boundaries, because both cells contribute to it. If these postulated substances lower the surface tension they will cause the two cells to bulge out towards each other. If they touch, capillary action may then draw them nearer together.

A similar phenomenon has been observed when the cells of adult sponges have been first separated by squeezing the sponge through a fine cloth. H. V. Wilson ('07, '11) who first made this experiment, observed that the isolated cells, after falling to the bottom, come together in twos, threes, etc. Groups of cells are produced, and if these are large enough a new sponge may regenerate. Galtsoff ('25) has more recently studied the process in greater detail. He has observed the same reunion of the isolated cells, and has added the important fact that a new sponge develops only when the aggregate contains cells of each kind of tissue of which the sponge is composed. Huxley ('21) has made similar observations.

CHAPTER XI

THE LOCALIZATION OF THE MEDIAN PLANE

The eggs of many animals appear under the microscope to be radially symmetrical around a primary axis. This axis is an imaginary line extending from that region on the surface at which the polar bodies are given off (pole) through the center of the egg to the opposite pole (antipole). Sometimes the pole is indicated by the distribution of pigment, or by some structure at the surface. The materials of the egg are stratified, or graded from the pole to the antipole.

Other eggs have a bilateral structure. These eggs are always enclosed in a thick coat or cuticle (insects, squid). The median plane of the embryo corresponds to the plane of symmetry of these eggs, but whether an innate bilateral structure of the protoplasm has determined the bilateral shape of the egg, and hence that of its coat also, or whether the shape of the egg-coat, determined by the parent, gives shape to the enclosed protoplasm, has never been determined. It is true that many references could be gathered from the literature to show that embryologists have not hesitated to assume, at times, that there is present in all eggs, before fertilization a real bilaterality, whether they show it or not in their external form. Other statements could be cited that seem to mean that a bilateral structure (or "principle") exists in the egg-cytoplasm which is the determining factor in locating not only the first planes of cleavage, but also the plane of bilaterality of the embryo. But how such bilaterality could direct or determine the position of cleavage furrows has never been explained, nor has it ever been made clear how such a postulated bilateral structure, were it present in the egg, could bring about the later differentiation with reference to a median plane.

It has been found that the early divisions of the egg may sometimes bear a definite relation to the planes of symmetry of the embryo. It is, therefore, necessary to examine thoroughly

the evidence that bears on this relation in order to find out whether the location of the cleavage planes determines the position of the embryo on the egg, or whether its position is independent of the cleavage.

The Relation of the Cleavage Planes to the Axes of the Embryo

The earliest observation showing the coincidence between the first plane of cleavage and the median plane of the body of the embryo was made by Newport in 1851. The position of the first cleavage in a frog's egg was recorded. The egg was left undisturbed until the neural folds appeared. Newport found that the median line between the folds corresponded with the mark indicating the plane of the first cleavage. The observation has been repeated by at least ten later observers, who have, on the whole, confirmed Newport's observation. This relation can be determined only if the egg remains entirely undisturbed during the 40 to 60 hours between the first cleavage and the appearance of the neural folds. As a matter of fact, the egg does not remain stationary throughout this time; for, during gastrulation the center of gravity of the embryo shifts so that the egg rotates. If, however, as appears to be the case, the egg rotates in the plane of the first cleavage (or other plane of symmetry), the original orientation may still hold; but if there are any irregularities in the process of gastrulation, these might cause the embryo to shift out of line. When the neural folds have been distinctly outlined, the embryo develops cilia over its surface that cause it, henceforth, to rotate within its membrane, and from this time onwards there is ample opportunity for a change in position. The record of the position of the median plane must be made, therefore, as soon as possible. When all these precautions have been taken, it is found that there are still exceptional cases in which the two planes in question do not coincide. This was first observed by Roux ('85), then by Schultze ('87), Hertwig ('94), Morgan and Tsuda ('94), Kopsch ('00), Brachet ('04). The later observations have shown that when the first plane of cleavage does not cut through the middle of the gray crescent (see below), the median plane of the embryo corresponds with the crescent rather than with the first cleavage plane. The experiments of Brachet in which one of the

first two blastomeres was injured (in cases where the first plane of cleavage did not coincide with the median plane of the crescent) confirmed this conclusion. It appears, therefore, that it is not the first cleavage itself that introduces a bilateral basis for the later development, but, on the contrary, the bilaterality of the frog's egg is already determined by the median line of the gray crescent which appears soon after fertilization, and before the first cleavage appears. Therefore, the question of fundamental importance is to determine what factor in the egg of the frog is responsible for the appearance of the gray crescent on one side of the egg (Figs. 41 and 43). There is, to be sure, still the

FIG. 41.—*a*, section of frog's egg through the crescent in plane of first cleavage. The penetration path of the spermatozoön is seen on the side of the egg opposite the crescent; *b*, surface view of frog's egg dividing into two. The cleavage cuts through the crescent. (After Schultze.)

problem as to why the first plane of cleavage generally cuts through the middle of the crescent, but for our present purpose this is a question of secondary importance.

THE FIRST CLEAVAGE PLANE IN THE EGG OF THE FROG

The median plane of the embryo of the frog is first indicated by the gray crescent of the egg. The first account, that of Roux ('88), described the crescent (Fig. 41) as arising after fertilization on the side of the egg opposite to that at which the sperm entered. It arises, he thought, by a withdrawal of some of the pigmented protoplasm from beneath a part of the black rind. Schultze ('00) also described the crescent as arising in the same way. He did not believe its localization was determined by the point of entrance of the spermatozoön, but that it appeared in a preformed region of the egg.

Morgan and Tsuda ('94) and Morgan and Boring ('03) described the gray crescent and its relation to the earlier division of the egg, but did not describe its origin. Brachet also is noncommittal as to the way the crescent is formed except that it is due to withdrawal of material at the edge of the black field, but he furnishes strong evidence in favor of Roux's contention that it lies on the side of the egg opposite to that of the penetration path of the sperm. Moszkowski ('03) ascribed the origin of the crescent to gravity, and stated further that if the constant influence of gravity is removed (by rotating) at the beginning of development, normal development does not take place. The disproof of this claim of Moszkowski was furnished by Kathariner ('01, '02), and by Morgan ('02, '04), while Roux, Morgan and

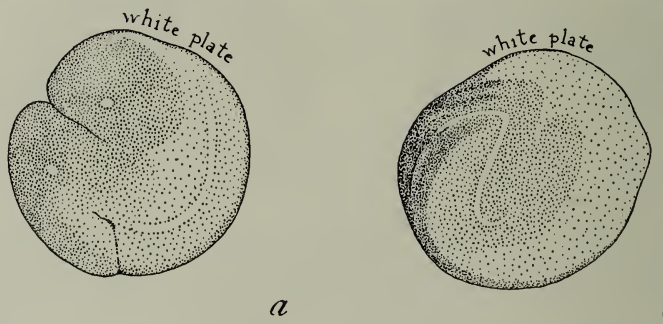


Fig. 42.—Cross-sections of frog's eggs that had been partially inverted and held in that position until the first cleavage appeared. As a result of the movements of the yolk towards the lower hemisphere a whitish plate appears on the surface that resembles the gray crescent of the normal egg. (After Schultze.)

Brachet have pointed out certain general relations that make Moszkowski's view highly improbable.

Roux, as stated above, first showed by localized fertilization that the first cleavage plane generally passes approximately through the point of entrance of the sperm. He also described the egg as sinking downward toward the side at which the sperm had entered and later he showed that the gray crescent appears on the opposite side by the withdrawal of some of the pigment from beneath the dark rind of surface pigment. In consequence, this region changes color to a slight extent—i.e., it becomes gray where formerly it was black (Fig. 41*a*, *b*).

It is true, as Roux pointed out, that a *somewhat* similar surface change may be induced, if, before cleavage, the egg is forcibly

held with the black hemisphere to one side. Under these circumstances the lighter pigmented material of the polar hemisphere may move under the influence of gravity toward the surface now uppermost and make the whiter edge somewhat darker. But the resemblance of such a light place to the gray crescent may be misleading. Born's experiments with inverted eggs gave somewhat the same sort of color change in the upper white field (Fig. 42).

Bambeke ('70) had seen that when the sperm penetrates into the interior of the frog's egg, it carries in with it a train of pigment that remains for a long time and serves to mark the penetration path of the spermatozoön. The presence of this path enabled Roux and Brachet to determine by means of sections that the gray crescent lies opposite to the penetration path, or approximately so. Thus in Fig. 41*a*, the sperm has entered above and to the right, and the gray crescent is opposite on the left side. The two sides of the egg at the time when the first cleavage appears are shown in Fig. 43*a*, *b*. One side, the future ventral, is darker than the other, the future dorsal side, in the middle region below the equator. The lighter side is the region of the original crescent. A two-cell stage as viewed below is shown in Fig. 44*a*. At the next cleavage each cell divides equally, giving two posterior lighter blastomeres and two anterior, darker blastomeres (Fig. 44*b*). After the third cleavage (Fig. 43*c*, *d*) the two sides of the egg are still unequally pigmented, and in some eggs at least (Morgan) the two upper black cells of the 8-cell stage, that are on the dorsal side, are smaller than the corresponding cells on the opposite side. In late cleavage stages (Fig. 22*h*, *i* and Fig. 43*e*, *f*), the cells on the dorsal side are a little smaller than those on the ventral side.

If this account of the relation of the crescent to the entrance point of the sperm be accepted, it follows that movements take place in the egg protoplasm immediately after fertilization that finally bring about a rearrangement of the material that determines, or is conducive to, the development of the future median plane of the embryo. Any meridian of the unfertilized egg may become the median plane of the embryo. In other words all meridians are equivalant before fertilization but not afterwards.

That there is still more to be found out concerning these conditions that determine the gray crescent will be evident from the following considerations:

It was pointed out by Morgan and Boring ('03), that there is great variability in the extent to which the gray crescent is developed in different clusters of eggs, each cluster coming from one female. If, as generally stated, the sperm may enter at any level in the upper hemisphere, it follows that the *extent* to which the crescent develops is not determined by the entrance point of

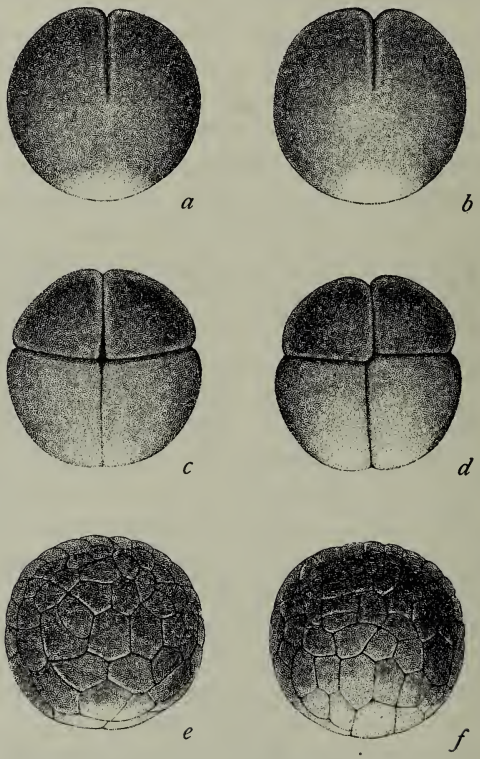


FIG. 43.—Pairs of drawings showing the opposite sides of the frog's egg during the early cleavages. The darker side, in the left-hand figures is the so-called ventral side of the egg, and the lighter side, in the right-hand figures, is the so-called dorsal side or the side of the gray crescent. In *e*, and *f*, the cells on the latter side are smaller than those of the opposite side at the same level. (After Brachet.)

the sperm, but rather by some property of the egg itself—perhaps the extent or amount of its pigmentation. As Brachet pointed out, this fact is a very strong argument against Moszkowski's view that gravity determines the crescent in the interval between deposition of the egg and the time it is free to rotate in the membrane after the sperm enters, for, since the eggs at first lie

in all possible positions with respect to gravity, the extent of development of the crescent should be very different even in the same cluster.

The movement of the surface material, with its contained yellow granules, in the egg of the ascidian Styela towards the point of entrance of the spermatozoön and the formation of the crescent on the same side of the egg might at first suggest that the yellow and the gray crescent are not comparable; for, according to the account here followed, they may seem to lie on opposite sides of the egg with respect to the entrance point of the sperm. A more careful study of the situation shows that

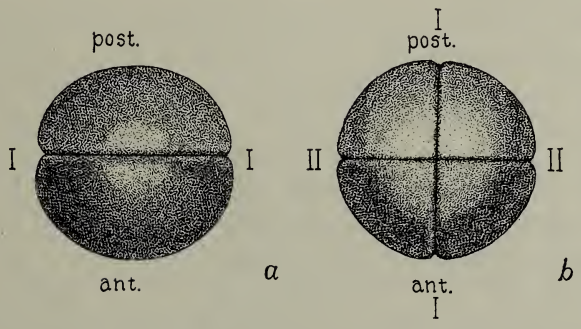


Fig. 44.—Lower pole of the egg of the frog at the two- and four-cell stages. The lighter side of these eggs, the side of the gray crescent, is above in these figures. In the left-hand figure the first cleavage plane (I–I) lies at right angles to the future median plane; in the right-hand figure the first plane coincides with the median plane, and the second cleavage has taken place. (After Brachet.)

this is not quite true. It is to be recalled that in the frog's egg the sperm enters in the polar hemisphere, and in the ascidian's egg in the antipolar hemisphere near the antipole. In the frog, the region where the sperm enters becomes the "ventral region" of the embryo, extending downwards to the posterior region which forms the tail, and in the ascidian the material of the yellow crescent forms the material from which the muscles of the tail develop. The material of the gray crescent in the frog becomes the chorda-mesoderm of the trunk in front of the blastopore, while in the ascidian the yellow crescent becomes the mesoderm of the tail. Thus to a certain extent, the two crescents finally contribute to the musculature of the embryos but to unequal amounts in different regions of the embryos. In this connection it may be recalled that in the frog's egg the crescent is just below

the equator, while in the ascidian's its location is nearer the antipole.

The first plane of cleavage of the frog's egg coincides in most cases with the middle of the crescent, but in a considerable percentage of cases there is no such agreement. Thus Morgan and Tsuda in 1894 found that in 119 eggs of Rana temporaria, examined at the end of the two-cell stage, the first cleavage cut the crescent nearly in the center in 76 cases, while in 30 cases the second furrow was nearer the center and in 13 cases it lay more nearly between. They pointed out that smaller cells were present later, on the lighter side of the egg, and that here the dorsal lip appeared.

A more detailed study of these relations was carried out by Morgan and Boring ('03) on the egg of Rana palustris. In 50 per cent of cases the first plane of cleavage coincided with the median plane of the gray crescent; in about 8½ per cent the second plane of cleavage coincided with the median plane of the crescent. In the remaining eggs, the first plane lay to one side of the middle of the crescent, but usually near to its middle. It was also recorded that at the 8-cell stage one or two of the upper black cells are smaller than the other members of this quartette, and it is always the cells that lie on the crescent side that are the smaller ones. The same difference was observed throughout the later cleavages to the time when the anterior end of the embryo develops on this side of the egg. This size difference is, perhaps, due to the crescent region having relatively less yolk. If so, its materials may have moved from other regions towards the side where the crescent develops, and presumably at the time of fertilization.

This view assumes that the crescent is formed by a movement of material to the future crescent side rather than to a withdrawal of material from that side as Schultze and Roux imply. Before discussing this question, further data relating to the coincidence of cleavage and crescent may be given.

In 100 eggs of Rana fusca, Brachet found perfect coincidence in 48 per cent and less than 10 degrees departure from coincidence in 20 per cent. The departure was between 10 degrees and 45 degrees in 10 to 13 per cent; it was 45 degrees in 10 per cent; and between 45 degrees and 90 degrees in 5 per cent. It was 90 degrees (i.e., at right angles) in 8 to 10 per cent of cases.

Jenkinson ('06) has also made some observations on the relation of the median plane and the first furrow and on that between the median plane and the plane of symmetry of the crescent. He confirms the earlier observation that the first two correspond in most cases, and that the latter two show a high coincidence. He found that the first furrow tended either to coincide with, or to be at right angles to, the plane of symmetry of the crescent.

These results show, as had the earlier ones of Morgan and Boring, that in the majority of cases the first cleavage coincides more or less nearly with the middle of the gray crescent. Two suggestions have been made to explain the failure of complete agreement. Roux thought that the penetration path sometimes failed to coincide with the copulation plane of the two nuclei and in these cases that the gray crescent, having been determined by the former and the plane of cleavage by the latter, the cause of disagreement was explicable. It has been suggested that handling of the eggs and the resulting distorting effects on them might shift the interior (or the spindle) and cause the later disagreement, but, so far, no one has furnished data to show that such changes occur. Until the actual physical movements that produce the gray crescent are better understood, it is not profitable to discuss further these suggestions.

It is necessary to point out a possible contradiction that runs through this literature. If, starting with Roux's original statement that the egg immediately after fertilization rotates as a whole towards the point of entrance of the sperm (depressing the "pole" on that side), then this must mean that the lighter material of the upper hemisphere of the egg is moved towards the side of the future crescent that is now higher up than before, if the new balance is to be maintained. The shift brings the margin of the light hemisphere above the equator on the side opposite the point of entrance of the sperm and the gray crescent should be produced there by the protoplasm of the upper hemisphere streaming in this direction. But Roux describes the streaming as taking place in the opposite direction to form the crescent; for, he says, the pigment is withdrawn from beneath the surface when the crescent forms.

If, as in the ascidian, the flow of the more fluid part of the frog's egg is toward the entrance point of the sperm, this should cause this side to rotate upwards and the end result would be

to bring the entrance point nearer the top of the egg. The withdrawal of the underlying pigment-bearing, top-protoplasm would leave the darker side, opposite the entrance point, lighter, as is said by Roux to happen, but the result as here supposed would cause a movement of the whole egg in an opposite direction from that which Roux postulates.[1]

The Location of the Median Plane in the Eggs of Other Amphibia

Jordan ('93) found that the first cleavage of the egg of the newt, Diemyctylus viridescens, is, as a rule, at right angles to the long axis of the embryo. The eggs are laid singly and are enclosed in a tough coat. The ovarian egg is spherical, but when laid the egg is nearly twice as long in one diameter as in the other. This shape must be imposed upon the egg either in its passage through the oviduct or by the surrounding membrane and jelly that are there added to the egg. The first cleavage is through the pole, and in the narrowest diameter of the egg. The egg becomes more nearly spherical as it divides. It becomes stuck to the bottom of the capsule where it remains until the gastrula lip appears. From the position of the lip, in respect to the elongated shape of the capsule, it is possible to make out the relation of the median plane of the embryo to the first cleavage plane. As stated, the two planes are at right angles to each other.

The sperm enters the egg about two hours after deposition; the two pronuclei come together from four to six hours later,—fertilization having taken place in the cloaca. The first cleavage begins ten to twelve hours after the egg is laid, dividing the egg into equal or nearly equal parts. The second furrow, at right angles to the first, appears 1½ to 3 hours after the first cleavage appeared. If the location of the first cleavage is determined by the shape of the egg it might also appear that the location of the median plane, at right angles to the first furrow, is determined by the compression of the egg. On the other hand, if the egg

[1] In a recent paper by Weigmann (Zeit. Wissen. Zool. CXXIX 1927), in which the origin of the gray crescent is described in much detail, evidence is brought forward to show that the crescent arises by a loosening of the pigment on one side of the egg. If this is correct it will not be necessary to postulate extensive movements of the interior of the egg as had been done by the earlier writers referred to in the text.

had a bilateral structure before fertilization, the egg might orient to the compression so that its pole is against the wall and the antero-posterior axis in the long axis of the tube. Neither view has any evidence for or against it. A third possibility is that the egg after fertilization rotates in its cylindrical membrane so that its antero-posterior axis is placed lengthwise to the confining membranes. But again there is no evidence for such an interpretation.

It has also been found in the eggs of several species of Triton, that the second cleavage plane coincides, as a rule, with the median plane of the embryo, but in a considerable percentage of cases it is the first plane of cleavage that coincides with the median plane. In none of these cases has a gray crescent been observed,[2] although it is possible that movements similar to those which produce it may take place but not be visible on the surface. By

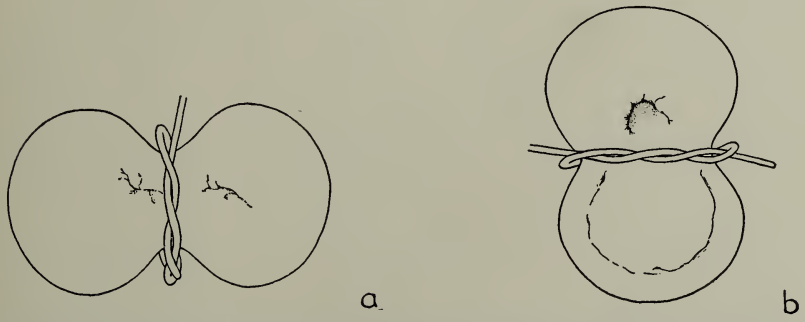


FIG. 45.—*a*, gastrula stage of the egg of Triton which had been constricted at the two-cell stage by a hair in the plane of the future median plane of the embryo; *b*, a similar egg that had been constricted at the two-cell stage, but in which the first plane of cleavage was frontal, i.e., at right angles to the median plane which is the more usual position of the first plane in the egg of Triton. (After Spemann.)

tying a ligature around the egg at the time of the second cleavage and in that plane (Fig. 45*a*), Spemann found that double headed embryos can often be produced, owing to the partial separation of the material into two parts. By further constriction two whole embryos result which is not, as a rule, the case when the egg of the salamander is constricted in the first plane of cleavage. The result furnishes experimental evidence, that, in this case, it is the second cleavage, and not the first, that usually corresponds to the median plane of the embryo. On the other hand, in some eggs, in which the loop has been tied around the egg in the first

[2] Indications of a gray crescent have been later described in Triton.

cleavage stage, the dorsal lip of the blastopore appears in one half only, and the prospective plane is at right angles to the first cleavage plane. In this case, the half containing the dorsal lip forms a whole embryo. The other half does not gastrulate, and does not produce an embryo.

The Location of the Median Plane in the Eggs of Teleostean Fishes

It has been shown by Clapp ('91) and by Morgan ('93) that there is no definite relation between the first or second plane of cleavage of the fish's egg (Fig. 46) and the median plane of the

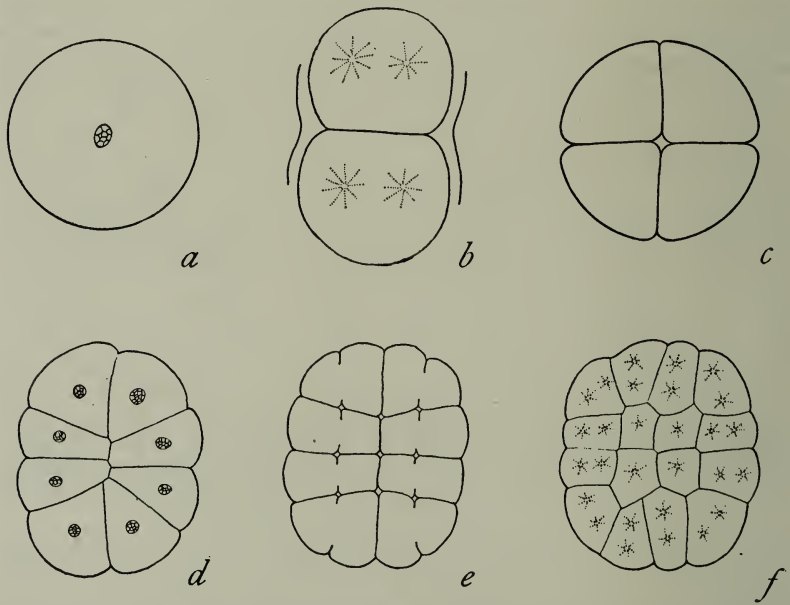


Fig. 46.—The first four cleavages of the egg of a teleost fish. (After H. V. Wilson.)

body. Miss Clapp determined this for the large fixed egg of the toad-fish, and Morgan for the small pelagic eggs of Ctenolabrus, and of Serranus. The floating, pelagic egg seems to be perfectly spherical, nor is there any plane of symmetry visible in the other, larger fish eggs. What factor or factors determine the median plane of the embryo is entirely unknown. The two-, four-, and eight-cell discs (Fig. 46*b*, *c*, *d*), are at times bilateral

in different planes, but this is apparently without significance for the later embryo. The many-celled disc of later stages appears to be again radially symmetrical.

For the toadfish egg Miss Clapp records that out of 23 cases, only 3 showed a coincidence between the first cleavage and the median plane; there was no case in which the second cleavage coincided with the median plane. For the egg of Ctenolabrus, Morgan records coincidence with the first cleavage plane in 5 cases, with the second in 2 cases; and with neither in 2 cases. For Serranus, coincidence occurred with the first cleavage in one case, with the second in 2 cases, and with neither in 2 cases.

The Origin of the First Plane of Cleavage in the Sea-Urchin's Egg

In the egg of one of the sea-urchins, Toxopneustes, it has been shown by Wilson and Mathews ('95) that the first plane of cleavage corresponds "in the great majority of cases, at least" to the entrance point of the sperm. Boveri has shown that the first plane also passes through the pole of the egg which can be identified at this time by the funnel in the jelly at the pole.

Wilson's observations were made by placing the eggs in a drop of sea water, over which a cover-slip supported by wax legs was placed. Sperm was then introduced at one side of the drop by means of a pipette, and the preparation placed under the microscope. In those cases where the sperm entered at one side of the egg, the point was noted by a mark on the slide. When the first cleavage appeared, it was found to coincide with the recorded point of entrance.

The relation between cleavage planes and median plane of the sea-urchin embryo has been variously described by different observers. Boveri ('01, '02, '05) concluded that the first cleavage coincides with the median plane, because, if one of the first two blastomeres is pathological, a defect appears on one side of the larva. He also observed in cases of "partial fertilization" that one half of the larva had small haploid cells, the other half diploid cells. He therefore inferred that the median plane corresponds approximately with the plane of the first cleavage.

Driesch ('00, '02, '06, '08) on the other hand, concluded that the second cleavage plane is the median plane. He placed two

cell stages of Echinus in dilute sea water which caused them to elongate at right angles to the first cleavage. The change in shape was sometimes retained until the gastrula stage when the median plane was found to be at right angles to the plane of the first cleavage. Boveri pointed out that the deformation may have changed the position of the median plane in the eggs.

Runnström ('14, '19) found that isolated ½ blastomeres of Paracentrotus are more fully developed on one side than on

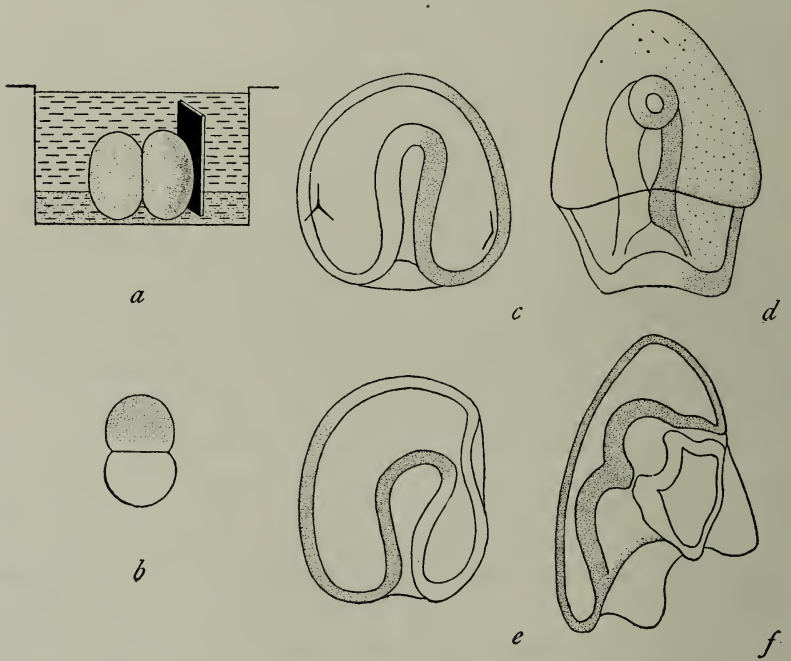


Fig. 47.—Method of staining the egg of a sea-urchin in the two-cell stage, *a* and *b*, by means of a block of agar, colored with Nile-blue. Later stages of gastrulæ and young plutei from such eggs, *c–f*. (After v. Ubisch.)

the other, and pointed out that the result is in agreement with the view that the first cleavage coincides with the median plane.

More recently von Ubisch ('25) studied the problems by means of intra-vitam staining. Three species of sea-urchins were used, and gave consistent results. Eggs in the two-cell stage were brought in contact with small plates of agar-agar that had previously been soaked in Nile-blue (Fig. 47*a*). The part of the egg in contact with the plate absorbed some of the blue. When one blastomere was partly colored, the egg was removed. The color

did not diffuse to any extent further into the egg during its cleavage. When, as in the above case, one blastomere was blue, the majority of the later embryos were stained on one side (Fig. 47*c*, *d*). In these cases the first cleavage coincided with the first plane of cleavage. In other cases, however, the anterior or posterior side of the embryo might be stained (Fig. 47*e*, *f*). In the latter the first cleavage plane could not have corresponded to the median plane. In other cases still, the stained region was in some intermediate part of the embryo. Von Ubisch concluded that

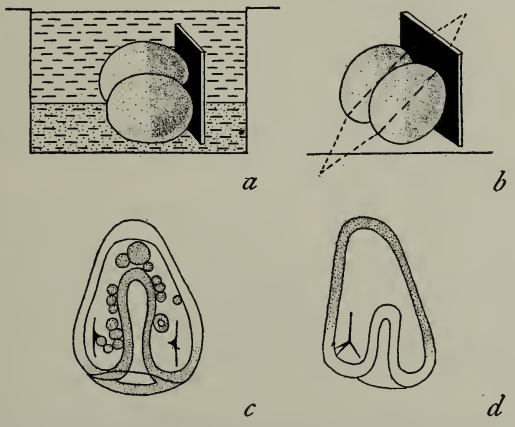


FIG. 48. *a* and *b*, eggs of the sea-urchin stained in the same way as those in Fig. 47, but in a different plane; *c* and *d*, later gastrula stages of same. (After v. Ubisch.)

there is no fixed relation between the median plane of the embryo and the first cleavage plane. As a matter of fact, development of whole embryos from isolated ½ blastomeres had already shown that there is no inevitable relation. To what extent the treatment of the eggs by Nile-blue in von Ubisch's experiments may have affected the result is not clear.

In the following table (Table XV) the numerical results are

TABLE XV

Color	Echinus esc.	Echinus mil.	Echinocyamus
Right or left half side	19	7	5
Dorsal	7	1	4
Ventral	6	1	6
Clearly oblique	4	2	2
Not clear (e.g. only one quadrant)	2	1	2

given. Taken as a whole the outcome confirms, I think, Boveri's view that, as a rule, the first cleavage coincides with the median plane of the embryo.

Von Ubisch also placed eggs in the two-cell stage against the agar plate in such a position that one side of both blastomeres touched the plates and became stained (Fig. 48*a*). Since the eggs are spherical at this time it is not possible to foretell, even if the planes were already determined, what part of the eggs are stained except in so far as the resulting embryos should be bilaterally or terminally stained if the first cleavage plane coincides with the median plane. The cases reported are not numerous, but it is evident that while some of them are more or less in agreement with the expectation stated above, a few showed the stain on one side (Fig. 48*b*, *c*). In the latter cases, at least, the agreement between the planes fails.

On the whole the results of von Ubisch are, I think, in fair agreement with the view that the first cleavage plane of the sea-urchin's egg usually coincides with the median plane, but that the relation is not "fixed." This may mean either that in some eggs the first cleavage fails to agree with a prospective median plane (assuming such to be present) as in the frog's egg, or that while the two planes usually coincide they may easily become shifted by external agents. That the echinoderm egg can develop a new median plane when extraneous conditions change it (as by isolating the blastomeres) has generally been accepted as proven.

The Origin of the First Cleavage Plane in the Eggs of Ascidians

In one of the ascidians, Clavelina lepadiformis, Van Beneden and Julin ('84) pointed out that the first cleavage divides the egg into equal blastomeres (Fig. 25*b*) which give rise to the right and the left half of the embryo. They also pointed out that this bilaterality was foreshadowed by the position of the segmentation nucleus and its spindle that comes to lie "posterior" to the primary axis (Fig. 25*a*). Other observers (Seeliger '85, Chabry '87, Castle '94, Conklin '05), have confirmed the relation between the first cleavage and the median plane of the embryo. In another ascidian, Styela (Cynthia) partita, Conklin ('05) has found that the median plane is foreshadowed even before the two pronuclei

meet (Fig. 28). Immediately after fertilization, movements take place in the cytoplasm that give a new arrangement to materials that have had a radially symmetrical distribution up to this time. The spermatozoön enters near the antipole, and the surface protoplasm in which lie imbedded yellow granules begins to flow toward the antipole to form there a yellow cap. Within fifteen minutes it has reached nearly to the lower pole, while at the opposite pole, where the polar body is about to be extruded, a slaty gray material is present that is rich in yolk. The sperm-nucleus and its aster move towards one side, which becomes the future posterior side of the embryo (posterior lip of blastopore). At the same time, the pigmented protoplasm also moves towards this side, and in such a way that the yellow cap is transformed into a superficial band, or crescent, lying below the equator of the egg on the posterior side with its horns extending about half way around the egg. The ventral border of the crescent is deeper in color than the dorsal border.

At this time, or later, other regions of the egg are faintly outlined. Of these the most significant is another crescent-shaped region opposite to the yellow crescent, i.e., on the anterior side of the egg. It subsequently passes into the cells that give rise to the neural plate.

Conklin is inclined to believe that the bilaterality of the egg, which does not become evident until the yellow crescent appears, is predetermined in the unfertilized egg, and that the sperm nucleus moves toward this predetermined side as it leaves the point of entrance and passes toward the equator. The main evidence adduced for this interpretation is that the sperm does not always take the more direct path toward the equator, but when it has entered to one side of the antipole it may at times cross the axis to another side. This evidence is undoubtedly important but calls for further examination. It would be significant, perhaps, in this connection to discover what factor determines the path taken by the egg-nucleus, and whether its movement and that of the sperm have an influence on each other.

The cleavage of the ascidian's egg takes place with almost no variation in the position of the successive planes of division and in the sizes of the resulting cells. In this respect it differs from the frog's egg, in which there is much variation, especially after the third cleavage. Since there are no sufficient observations

as to the results that take place when the first cleavage of the ascidian fails to coincide with the crescent, we have no means of deciding whether the crescent or the cleavage plane is the basis for subsequent bilaterality. This question is further discussed in Chapter XVII.

The Location of the Median Plane in the Eggs of Nematodes

The eggs of many nematodes are elongated and enclosed in a thick membrane (Fig. 49). In Ascaris (Rhabdonema) nigrovenosum, there is a micropyle at one end through which the spermatozoön enters. The egg is probably symmetrical around its long axis. The polar bodies are given off at one end of the egg (inside the membrane), and the spermatozoön enters through

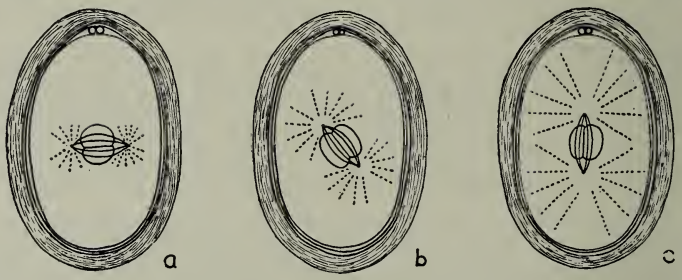


Fig. 49.—Movement of the segmentation spindle through 90 degrees before the first cleavage in the egg of Ascaris nigrovenosum. (After O. Hertwig.)

the micropyle at the opposite end. Whether the shape of the egg is imposed upon it from without, as, for instance, by the shape of the tube in which it lies at the time when the membrane is formed, or whether the tube forms the membrane and in this way determines the shape of the egg is not known, but from analogy with the eggs of the Ascaris of the horse it seems more probable that the egg itself produces the membrane, and if so, then the shape of the egg however caused, must, at the time, determine the shape of the membrane. Later when the membrane has hardened, and the egg escapes from the oviduct, the membrane keeps the egg in the shape it had at the time when the membrane was formed.

The axis of the egg coincides with its long axis; for, the polar bodies are given off at the end of the egg opposite to the micropyle.

The sperm brings in the centrosome or at least one develops near the sperm head. As the sperm-nucleus approaches the egg-nucleus, the centrosome divides, and a spindle appears between the two centers. The spindle lies at first across the egg in the plane of apposition of the two pronuclei (Fig. 49*a*), but before the division of the cytoplasm begins the spindle rotates and its axis comes to coincide with the long axis of the egg. The nuclei rotate at the same time (Fig. 49*b*). The first cleavage comes in across the equator of the egg (Fig. 49*c*).

The axis of the egg of Ascaris megalocephala in relation to the first cleavage has been studied by Boveri ('10) and by zur Strassen ('96, '99, '06) and more recently by Schleip ('24). The immature eggs during the growth period are attached around a central axis (rhachis) and are closely appressed against each other. Each is in form a long cylinder. When nearly full size, the eggs become detached, round up, and lose all trace of their axis of attachment. The non-motile amœboid spermatzoön fuses with the naked egg-cytoplasm, and a thick membrane is then secreted by the latter. The first polar body is given off, and remains sticking to the inner surface of the membrane. The second polar body is next given off, and sticks to the surface of the egg. Meanwhile the egg shrinks away from the inner wall of its membrane. When the cleavage spindle forms, it may point in any direction with respect to the second polar body, and the first cleavage plane likewise bears no definite relation to the location of the polar body at that time. If the second polar body is assumed to lie at the pole and to retain its position there, then the cleavage really bears no relation to the axis of the egg. There is, however, evidence that the polar body does not remain in the place where it is given off. There is other indirect evidence that the polar bodies are both given off at the pole, and that the first cleavage plane is, as a rule, at right angles to the primary axis of the egg.

Both Boveri and zur Strassen have discussed the question of "the polarity" of the egg of Ascaris. If I understand zur Strassen's ('06) view, he thinks that the polarity is something that comes with the "organization" that develops before the first cleavage. Nevertheless he does not regard the egg as itself isotropic. Boveri agrees with zur Strassen that polarity is not present in the first oöcyte stage, but is something new that

develops at maturation. From experiments with the centrifuge he decided that the first division is related to the point of extrusion of the polar bodies. These somewhat vague interpretations appear to be in part related to the attempt that zur Strassen and Boveri have made to interpret the twin eggs of Ascaris. These questions will be referred to again in connection with the fusion of two eggs to form one embryo.

Schleip ('24) has described eggs, from a certain female, that were pear-shaped. In most of these eggs the second polar body had been given off at the blunt end, and the first cleavage is at right angles to the long axis of the egg. It is true that the eggs of this female were in some way abnormal, but it seems not improbable, Schleip thinks, that for the most part they kept, to some extent, their original elongated shape that they had when they were attached to the rhachis, and in consequence furnish evidence of the relation of the axis of the egg to the plane of cleavage. It is to be kept in mind also that there has never been any cogent reason for supposing the egg of the Ascaris megalocephala to be different in these fundamental aspects from the eggs of other animals.

The study of the cell-lineage of the Ascaris megalocephala has made clear the relation between the median plane of the embryo and the planes of cleavage. The first cleavage plane divides the egg into two equal or sub-equal cells (Fig. 13), one of which, AB, is poor in yolk, the other P_1 rich in yolk. The second cleavage in AB is at right angles to the first, and divides the cell AB into two equal parts, A and B (Fig. 13*b*). The second cleavage is parallel to the first in P_1, dividing it into two equal parts, EMSt and P_2. The four resulting cells have the T-shaped arrangement shown in Fig. 13*b*. At this stage there are two planes of symmetry present at right angles to each other, one cutting through the middle of all four cells, the other plane cutting through EMSt and P_2, but between A and B. The former corresponds approximately to the future median plane.

The four cells now begin to shift, the two cells EMSt and P_2 bending so that P_2 comes in contact with B as seen in Fig. 13*c* to form a rhombus. Since at this time A and B are identical in appearance it is perhaps arbitrary to assume that P_2 comes in contact with B rather than A. In other words, if the lower arm bends to the left, P_2 and B come in contact, but if to the

right then P_2 will make contact with A. But if at this time the antero-posterior plane is present (though not visible) it may be that the two cells EMSt and P_2 turn toward a predetermined side. The question will be further discussed below in connection with an experiment of Bonfig's.

The two cells, A and B, that are called the dorsal group, and contain most of the future ectoderm, next divide at right angles to the plane of the rhombus (Fig. 13*d*). The plane that lies between the sister cells lies in the future median plane of the embryo. It passes, as yet, through the middle of each of the two cells of the ventral group. It is not until a later cleavage that cells derived from these two cells come to lie to the right and left of the median line.

A shift now takes place in the four dorsal cells (Fig. 13*d*, a^1, a^2, b^1, b^2). This introduces an asymmetry into the cleavage pattern. There is some evidence that one of the asymmetries of the adult can be traced back to this change.

It is evident from this account of the early cleavage that neither the first nor the second cleavage planes coincides with the future median plane, but that the division of the dorsal cells (third cleavage) establishes for these cells a plane that corresponds with the future plane of symmetry, but still leaves the determining factor for the antero-posterior direction unaccounted for. It may, as stated above, be already predetermined in the egg, or may be determined by the direction which the two cells EMSt and P_2 take when the rhombus is formed. If the turning of the latter is due to chance, then an extraneous factor gives the antero-posterior orientation. This is what is suggested by experiments carried out by Bonfig; but even then it is to be noted that the turning is still postulated to take place in the plane of the cell AB.

Bonfig ('25) compressed eggs in the two-cell stage between two slides.[3] Some eggs came to divide between the compressing plates as shown in Fig. 50. If the spindle in AB is parallel to the compressing plates the four cells will after the next division have the normal arrangement. Other eggs come to lie between the compressing plates in such a way that the spindle in AB is at right angles to the pressure. When the elongation of the AB cell takes place as it is about to divide, the elongating cell shifts

[3] The first pressure experiments on the Ascaris egg were made by Girgoloff ('11).

so as to lie more or less parallel to the pressure. One of the possible configurations is to leave P_1 (and later its daughter cell EMSt) in contact with one of the dorsal group, A or B (Fig. 50*a*, *d*).

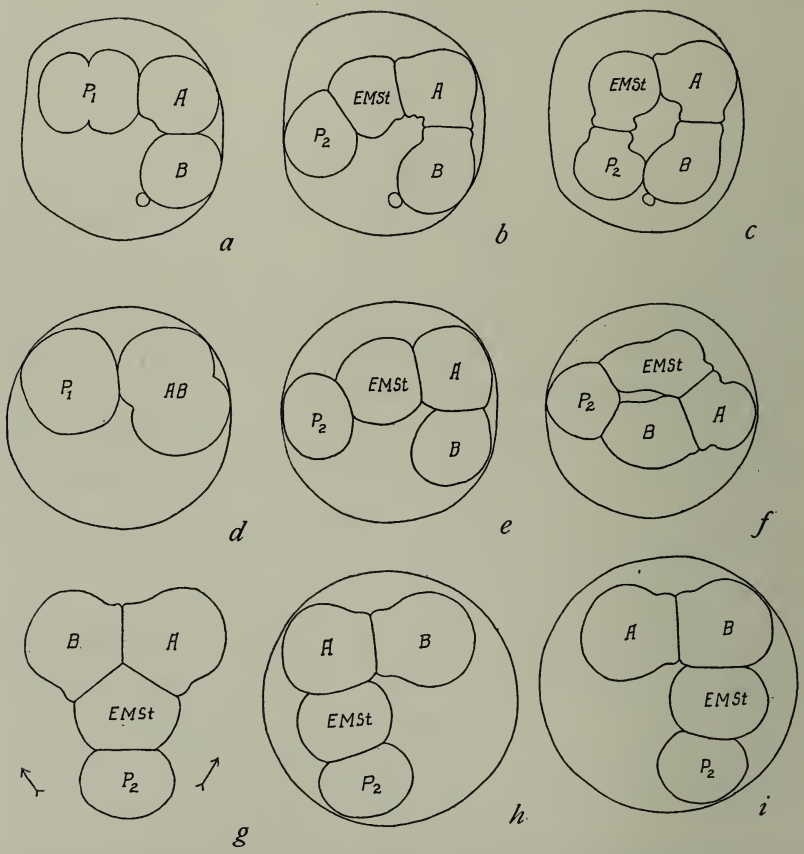


Fig. 50.—Cleavage of the egg of Ascaris megalocephala that had been compressed during the two-cell stage. The figure shows the changes that take place when the pressure has been removed. (After Bonfig.)

Unless there is some special condition that makes it more probable that P_1 sticks to the A-end of the AB cell (if there is such a distinction) rather than to the B-end, the subsequent contact formed between the two free ends (Fig. 50*h*, *i*) will be sometimes with A, sometimes with B. If so the antero-posterior axis is not predetermined.

The cause of the turning in the normal embryo to produce the rhombus is unknown, but two observations may be significant. First, A and EMSt change their form in such a way as to suggest, at least, that this is the cause rather than an effect of the turning, but, as Bonfig points out, the cell EMSt continues to elongate at this time, until P_2 comes in contact with the membrane and then the turning takes place. He thinks that the direction of the turning (in an already fixed plane) is determined by the contact which makes turning in one direction easier than in the other, according to the position of the group inside the membrane. This means that an "accident" determines which shall become the antero-posterior axis of the embryo.

It may seem improbable that an egg whose divisions are as definite as this one should have the antero-posterior relation left to a chance-turning in the membrane, especially as they must still turn in a particular plane, i.e., in the long axis of the AB cells. On the other hand the result of Bonfig's careful observations seems to make it probable that if extraneous agents cause the reversal of the tongue of the T, the antero-posterior development of the dorsal group may be reversed to conform to the axis of the ventral group. Such an interpretation is not inconsistent with the potentialities of some other blastomeres. It may be added that after removal of the pressure, the exceptional eggs that Bonfig had observed, developed into normal embryos.

Bonfig has also examined the asymmetry of the cleavage pattern of Ascaris which first appears in the dorsal (ectodermal) group after the third cleavage. This is brought about by the shifting of the four cells, so that two of them that are vis-à-vis come together at a higher level across the potential median line where they form a cross furrow. The two other cells (also vis-à-vis) move to a lower level. Occasionally the pattern is reversed as can be seen by referring it to the P_2—EMSt line. There is a possibility that there is a connection between these cases of reversal and those found in the right or left posterior position of the large excretory funnel of the adult. Several suggestions have been made as to the origin of this asymmetry (zur Strassen, Boveri, Bonfig), but the problem seems as elusive as that of the reversal of asymmetry of the gastropod cleavage.

The Location of the Median Plane in the Eggs of Nereis

Just ('12) has shown that in the egg of Nereis the point of entrance of the sperm determines, in all cases where disturbing factors are eliminated, the point through which the first cleavage passes. The eggs, as soon as obtained from the body of the female, were placed in a single layer in sea water in which India ink had been previously ground up. Sperm was added. The moment a spermatozoön touches the egg membrane, the contents of the cortical layer of the egg begins to flow out as a viscid, transparent substance through pores in the membrane. It becomes the jelly of the egg. At the point where the head of the sperm is affixed to the egg, the outflow is interfered with. The secretion flows around the head, and then around the tail. Elsewhere the fine particles of India ink are pushed ahead of the jelly, so that each egg appears surrounded by a halo. The particles of India ink sticking to the head and tail of the sperm render their position visible in the jelly; a trail of ink reaches from the head of the sperm to the surface of the jelly. This trail remains after the sperm has entered and is visible until the cleavage appears. Special precautions must be taken to keep the eggs quiet, since they are free to move inside the membrane. It is also important to make use of only those cases in which the ink trail reaches the surface of the egg as seen in side view, for, if it lies above or below the equator, the last part of its path may be obscured and a false impression given as to the exact point at which the head entered. It may be recalled that the second plane of cleavage in Nereis corresponds more nearly to the median plane than the first, although neither the first nor the second can strictly be said to correspond exactly to the median plane. Nevertheless, since the median plane bears a perfectly definite relation to the first plane, the outcome in Nereis is the same in principle as in cases where the two planes are identical.

Eggs with Bilateral Symmetry before Fertilization

In addition to the cases in which the egg, before fertilization, appears to be radially symmetrical around its primary axis, there are other cases in which the egg has already a plane of symmetry when it is fully formed. In these, the median plane of the embryo

corresponds with the plane of symmetry of the egg. For instance, the egg of the squid has, according to Watase ('91) a strictly bilateral structure (Fig. 51a, b). The first division is in this plane as is later the median plane of the embryo. It has not been shown whether the shape of the squid's egg is determined, in the first instance, by the shape of the follicle which surrounds it, or whether it is determined by the protoplasm of the egg itself. It is also not known whether the first spindle places itself across this plane of symmetry, preparatory to the first division, in response to the shape of the egg, or whether other factors determine its position. Experimental evidence is lacking.

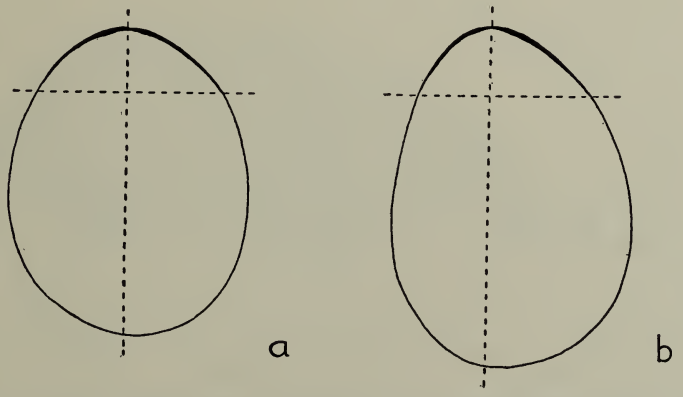


FIG. 51.—Squid's egg in plane of symmetry; and at right angles to that plane. (After Watase.)

The eggs of many insects have a marked bilateral form (Fig. 52) which, in a way, corresponds with that of the mother's body. The anterior end of the insect's egg is not the point of the surface at which the polar bodies are given off. These lie anteriorly, but on the dorsal side of the egg at a point generally not very far from the micropyle of the egg through which the spermatozoa enter (Fig. 52c). The plane of bilaterality of the embryo corresponds with that of the egg.

The cleavage of the insect egg is centrolecithal, that is, the segmentation nucleus first divides into two nuclei, and the daughter nuclei later divide again and again, but the protoplasm of the surface of the egg does not split up at this time (Fig. 22a, b). Only when a large number of nuclei have appeared, and have

reached the surface, does the layer of protoplasm that covers the egg become constricted around these nuclei into a large number

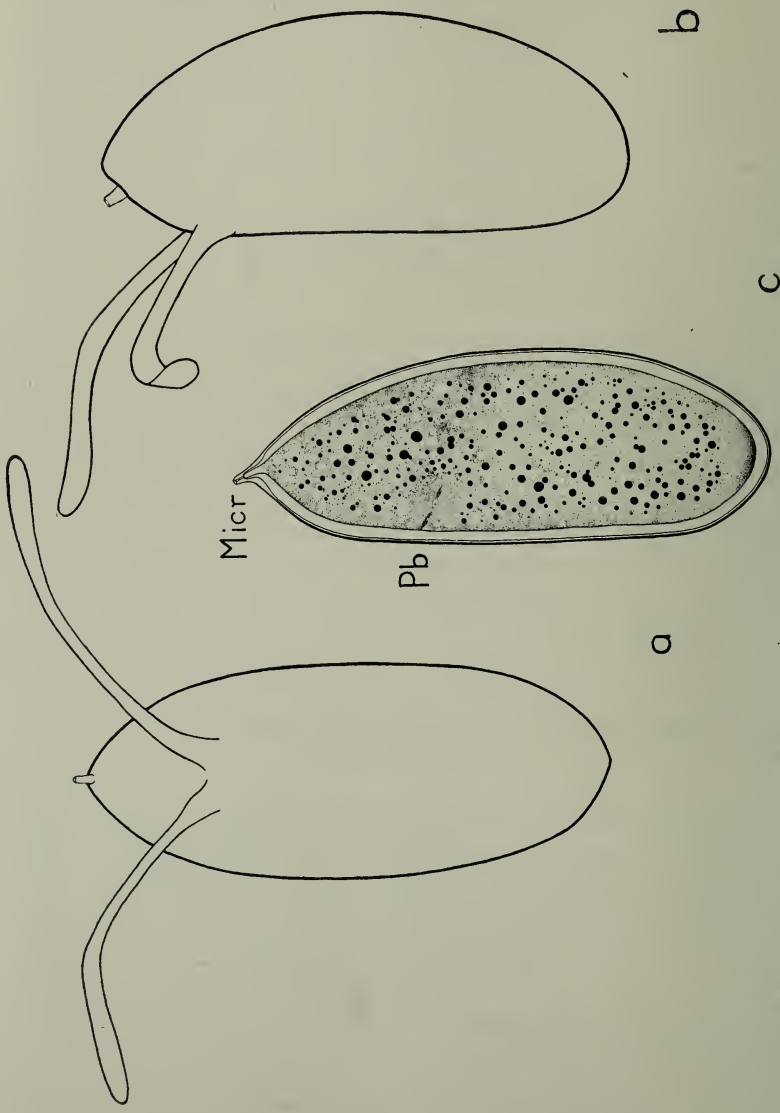


Fig. 52.—*a*, bilateral egg of Drosophila, as seen from dorsal side; *b*, from right side; *c*, section of egg in median plane and through the micropyle (*micr*). The polar bodies have been given off on upper third of dorsal side.

of cells. The bilaterality of the embryo proper can become apparent only when the embryonic organs develop out of this surface layer.

The Position of the Chick in the Egg

The determination of the median plane of the embryo of the hen's egg, and the position of the embryo with respect to the shape of this egg has aroused a great deal of interest and speculation. It has long been known that the young embryo chick lies, in most cases, across the long axis of the oval-shaped shell. If the large end of the shell is held to the left and the shell opened, the embryo is found on the upper surface of the yolk, with its head away from the observer in a great majority of cases (Fig. 53). The discovery of this relation goes back at least as far

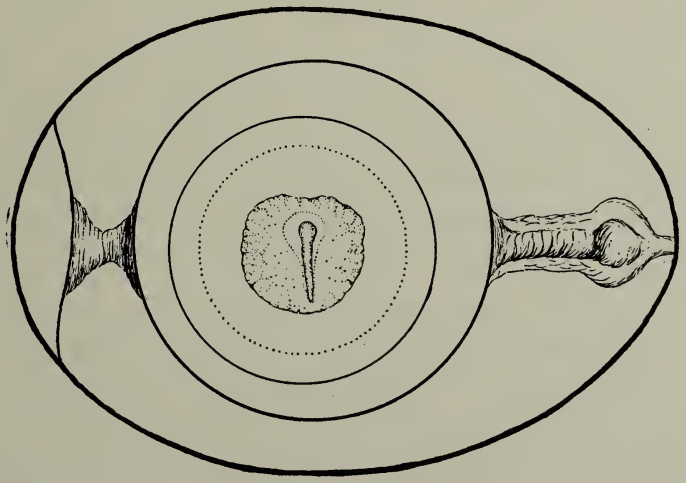


Fig. 53.—Diagram of hen's egg showing position of embryo across the long axis of the egg. (After Duval.)

as von Baer ('28) and was probably known before his time according to Bartelmez ('12, '18). The yolk on which the embryo lies is not spherical according to Bartelmez. Its longest axis corresponds with that of the shell. Its primary axis, extending from the embryo "downwards" through the center of the egg, is the shortest axis; while the axis in the third dimension ("horizontal") is intermediate in length. The yolk is surrounded by the spirally wound concentric layers of albumen, or "white," that have been formed as a secretion from the walls of the oviduct as the egg has passed down its length. The pointed end of the future "egg" is in advance, as the egg passes down the oviduct in a spiral path.

This end may therefore be called the cloacal end and corresponds approximately to the right side of the later embryo. When the egg enters the oviduct it receives first the cloacal chalaza and then the opposite chalaza; these are fastened to the vitelline membrane and hold the egg in position, except in so far as it rotates as it progresses on the axis connecting the chalazae—the future long axis of the yolk. In others words, the egg keeps its pole always against the wall of the oviduct and the path of the pole inscribes, as it were, a spiral path on the wall of the oviduct.

It may appear that the shape of the yolk is due to the compressing layers of albumen which in turn are compressed by the hard shell. If this is the case the position of the embryo across the long axis might be supposed to be due to the shape of the egg, but this hypothesis alone fails to explain why the head is nearly always turned towards the same side of the egg. Since the two ends of the egg are of different shapes, one broad (where the air space comes to lie), and one pointed, it might appear at first sight that the resulting pressure relations might account for the orientation of the embryo on the egg, but again this suggestion will not explain why the head is nearly always directed towards a particular side.

Bartelmez, who has studied the egg of the pigeon in great detail, where the axial relations are the same as in the hen's egg, believes that the egg is bilateral before it enters the oviduct, and that one particular side of the egg enters first and that this accounts for the orientation of the egg in the oviduct,[4] and also for the position of the embryo on the egg. In other words, he thinks that the ovarian egg has both a bilateral structure as well as a primary axis at right angles to this structure. His study of the early eggs, even before the yolk is laid down, led him to conclude that this bilaterality is expressed both in the excentric position of the nucleus (in regard to the primary axis) and in the elongation of the egg in one axis that corresponds to that seen in the oviducal egg, but the difficulties of such a determination must be too great to allow any such definite statements.

The egg in the ovary is enclosed in a follicle that projects from the surface of the ovary as the egg enlarges. The walls of the follicle develop a rich supply of anastomosing blood-vessels,

[4] Something more than bilaterality is required to explain why the right side rather than the left should be the first to enter the oviduct.

except over a crescent-shaped line, where the outer and inner layers of the follicle are fused with each other. This non-vascular crescent is known as the stigma, and according to Bartelmez the stigma lies nearly in the long axis of the egg. It develops, however, at a later stage than the supposed bilaterality of the egg, and he thinks follows it. According to Bartelmez a bilaterality is present even in the very tiny ovarian egg.

On this view it is, of course, necessary for consistency to suppose that at the time when the egg breaks out of the follicle, to be swallowed by the enveloping infundibular funnel, one of its ends enters first, as stated above, and the method of breaking of the stigma is supposed to be of such a kind as to bring this about, or else the materials of the ends must be supposed to be of such a sort that one end is more likely to enter than the other. Floating ovarian eggs, freed from the follicle, give evidence, Bartelmez thinks, that there is a difference in the specific gravity of the two ends in question. His account is so detailed and positive that it might appear to settle the question for the bird's egg; but on the other hand, it may be pointed out that the evidence relating to the very young eggs is by no means as certain as the importance of the situation demands. It is, moreover, difficult to obtain accurate evidence of this kind. The considerable variation in the position of the embryo with regard to the long axis of the egg, on which the author lays such emphasis, indicates either that the early bilaterality of the egg bears a very variable relation to the axis of the embryo, or else that there is much variation in the way the egg enters the oviduct and passes down its length, which is not in full harmony with the view by which the process is described as taking place. In the rare cases where the head of the embryo is turned in the reverse direction Bartelmez assumes that the wrong side of the egg has, perchance, entered the oviduct first.

It is certain that the antero-posterior axis of the chick embryo is already determined in the oviduct before the shell is laid down, since gastrulation takes place in the oviduct. The pressure of the oviduct on the two ends of the egg, which determines the shape of the albumen, would give, theoretically, an explanation of one of the factors involved, namely, the position of the embryo across the long axis of the egg. It remains still to be shown why the head of the embryo lies on a specific side of the yolk. The follow-

ing suggestion may furnish a clue. If the position taken by the egg on entering the oviduct is due to differences in compressibility of the egg of such a sort that it is more easily compressed in its primary axis (due to the distribution of the concentric rings of yolk); and if the advancing and the following ends of the egg are under somewhat different conditions (due to the contraction behind and the relaxation of the tube in front which allows the egg to advance); and if the spiral path taken by the egg in its passage down the tube is due to the structure of the oviduct; and if the spiral is, as a rule, a right- (or left-) handed spiral (as shown by the albumen), then the conditions are such that one side of the blastodisc is subjected to different influences from the opposite side. If this affects the position of the embryo, either directly or indirectly, in somewhat the same way as the bilateral and antero-posterior shape of the egg-case in many insects determines the orientation of the embryo, there is a formal explanation of the orientation of the chick. Further observations are needed to settle the truth or falsity of this hypothesis.

General Conclusions

From the preceding considerations it is evident that, in eggs that have, before fertilization, a bilateral form, there is at present no way of deciding whether the shape is due to an inherent property of the protoplasm, or is imposed on the protoplasm by the maternal cells that produce the egg-shell. On the other hand, in eggs that are radially symmetrical around the primary axes before fertilization, there is evidence to show that bilaterality is introduced at the time of fertilization. It has been shown, for example by Wilson in the egg of the sea-urchin, that the spermatozoön may enter at any point of the surface, and that its entrance determines the plane of the first cleavage, and in so far as the bilaterality of the embryo coincides with the cleavage planes, it follows that the bilaterality is induced from the outside. In Nereis it has been shown by Just ('12) that the first cleavage cuts through the point of entrance of the spermatozoön and since, as has been pointed out, the bilaterality has a definite relation to the cleavages, it follows here also that the bilaterality is superimposed from outside on a radial form. In the frog's egg it has also been shown by Roux ('85), Brachet ('03), and Jenkinson

('09) that the path of entrance of the spermatozoön coincides with the plane of bilaterality of the gray crescent (Fig. 41), and that this plane is also the plane of bilateral symmetry of the embryo. The gray crescent is not present in the unfertilized egg, but appears about an hour and a half after the sperm has entered.

These relations leave scarcely a doubt but that in these eggs —and they are typical of their kind—the bilaterality is induced from outside. I am inclined to think that this is also probable in the case of those eggs where the shell imposes on them from outside a bilateral form, although this is only an inference and has not yet been demonstrated by experiment or observation.

There are two further sources of evidence that relate to the origin of bilaterality in the frog's egg: (1) the origin of the gray crescent in eggs fertilized by two or more spermatozoa, and (2) the location of the crescent in parthenogenetic eggs. The former relation has been studied by Herlant ('11). If the unfertilized eggs of the frog are taken from the uterus and placed in concentrated sperm, many of the eggs are entered by two or more sperm. These sperm always enter in the black hemisphere apparently at any point. In dispermic eggs the gray crescent appears at the normal time and always between the two points of entrance of the spermatozoa, as shown in Fig. 7. Hence, as in the normal egg, there is a definite relation between the points of penetration and the gray crescent, and the result is, in a sense, a compromise between the two influences acting at the same time. Whether a normal embryo results, depends on special conditions relating to the division of the nuclei, but when an embryo does develop it stands in the same relation to the crescent as does the normal embryo.

When more than two spermatozoa enter an egg a single crescent also develops, but Herlant could not make out any relation between it and the entering points of the spermatozoa. Yet, in the light of the other evidence, it seems to me more probable that there is such a relation, than that, under these circumstances, the position of the crescent is predetermined in the egg structure itself, as Herlant suggests.

The origin of bilaterality in the parthenogenetic egg of the frog has been examined by Brachet ('12). The development was started by Bataillon's method of puncturing the egg with a fine

needle. In all eggs so punctured the gray crescent appears as in normally fertilized eggs, but it has no definite relation to the point of puncture. Only a small percentage of such eggs show a regular cleavage, and a still smaller percentage develop into embryos. In those that do, Brachet found that the embryo develops in relation to the crescent. He interprets the result to mean that the egg has a sort of labile bilaterality that may express itself in the egg alone, but which in normal fertilization may be altered into a new bilaterality by the entrance of the spermatozoön. In this respect he agrees, to some extent at least, with the older conclusion of Schultze, that the unfertilized frog's egg has an inherent bilateral structure; but Schultze also thought that it is not changed by the entrance of the spermatozoön. Personally, I think that one is not obliged to draw the conclusion from Brachet's results that he has drawn. On the contrary, I am more inclined to think that the formation of the crescent in the parthenogenetic egg may have some definite relation to the internal changes connected with the development of the artificially induced cytasters that produce the mitotic figure for the first cleavage. Herlant ('13) has studied the origin of these cytasters and has shown that two or more develop in or near the path of entrance of the needle. Two of them, under optimum conditions, serve as orienting points for the cleavage spindle. Their presence may possibly induce the internal changes that locate the crescent. It is true that this cannot be shown to be the case at present, but in the light of the other evidence relating to the origin of bilaterality in the frog's egg, I am inclined, at least, not to ignore such a possibility.

Brachet ('04) examined the effects of injuring the blastomeres of eggs whose first plane of cleavage does or does not coincide with the median plane. Eggs in which the first cleavage had passed through the middle of the gray crescent were injured in one blastomere by a hot needle. Half-embryos, like those described by Roux, developed (Fig. 153*A*). Other eggs, in which the first cleavage had cut the egg parallel to the gray crescent, were injured by the hot needle in one or the other blastomere. If the blastomere that does not contain the gray crescent is injured, the dorsal lip appears on the uninjured blastomere in the normal position and extends around the yolk as far as the dividing line between the halves. The closure may be interfered

with by the undeveloped half, but the anterior end of the embryo develops on the developing half (Fig. 154*d*).

If the blastomere with the crescent is injured, the remaining blastomere does not produce an embryo. It has been doubtfully stated by Roux that the ventral lip of the blastopore, in this case, appears on the uninjured half.

In other cases, the first plane of cleavage may pass in neither one of the two preceding planes, but somewhere between the two, producing one cell that contains most of the gray crescent (including its central part) and another that contains only one side of the crescent. If the half without the crescent is injured an almost complete anterior end develops from the uninjured blastomere. The converse operation is not described. These results, taken in connection with other evidence, show that the development of the isolated blastomere of the frog has the capacity to develop either into a half or into a whole structure according to the conditions that prevail at the time when the embryo proper begins to take shape.

CHAPTER XII

THE CHROMOSOMES OF THE EGG AND THEIR DIVISION

The difficulties of dealing experimentally with the chromosomes by direct means have proven almost insuperable. As yet even the fine technique of micro-dissection has yielded very meagre results with the chromosomes, and the centrifuge has not furnished a means of separating the individual chromosomes that hold together and move, if they move at all, as a group. Almost the only recourse left to the experimentalist is to look for accidental irregularities in the distribution of the chromosomes in the egg and sperm, and then to note the resulting effects on the embryo. With a start of this kind geneticists have been able to get valuable evidence concerning the relation of the individual chromosomes to the general situation, and embryologists have also taken advantage of similar situations and reached certain conclusions. An analysis of these cases has also thrown some light on the significance of chromosomes in development.

Changes in Size of the Chromosomes During the Early Cleavages and Development

During the cleavage of the egg at normal temperatures, the chromosomes may divide at about the rate of once every hour for ten hours or longer. Each cell gets a full set of chromosomes, but the size of the resting nuclei steadily becomes smaller. The size of the nuclei of the blastomeres may be stated, in general, to be proportionate to the size of the cells. In cases where one daughter set of chromosomes goes into a small cell, and the other daughter set into a large cell, the nuclei are of different sizes, although each contains, at first, the same number of the same sized chromosomes. It is not improbable, here, that the size of the nucleus is determined by the amount of the more watery substances available in each cell that constitutes the bulk of the nuclear sap.

At each cell-division the chromosomes split lengthwise into exactly equivalent daughter halves. A resting stage follows, and the process is repeated. Unless rapid growth of the chromosomes takes place during the resting stages they would become reduced to a very small fraction of their original width as a result of the successive divisions, and at the end of the cleavage period the total volume of chromatin would be the same as at the beginning. But, if after each division the chromosomes grow to their original size before the next division, as is generally assumed to be true, the total amount of chromatin at the end of the cleavage stage would be enormously increased. The evidence shows that neither of these alternatives is strictly true so far as the measurable size of the chromosomes is involved, but it is the width of the chromosomes rather than their length that is involved in the above comparison and unfortunately it is their length that has in most cases been measured. From the genetic point of view, the length may not seem to be a matter of great importance, if, as the genetic evidence assumes, each gene is divided at each division and all the cells have the totality of the genes. As yet we do not know how their arrangement is affected by the length of the chromosomes.

Erdmann ('08) has made several series of measurements of the chromosomes, as well as of the nuclei and cell-volumes of Strongylocentrotus lividus, from the two-cell stage to the pluteus. She also compared the results in three series, reared at three different temperatures. The results in the normal culture (15–16 degrees C.) are summarized in Table XVI.

The table shows that, with the decrease in size of the blastomeres, there is also a decrease in the "size" of the nuclei. Similarly there is a steady decrease in the "size" of the individual chromosomes. At the pluteus stage the chromosomes have only 1/40 the volume of the chromosomes on the first spindles. It has, however, been found by Baltzer ('09) that there is little or no decrease in size of the chromosomes during the first and second divisions of Strongylocentrotus lividus and of Echinus, and Godlewski ('08) has shown that the size of the nuclei in the 2, 4, 8, 16, 32, 64 blastomeres of Echinus is about the same. Later, the nucleus decreases rapidly in size. He infers that the chromosomes undergo no considerable change in size during the whole development. He points out further that, even with the reduction

TABLE XVI

NORMAL CULTURE 15°–16° C.

Stage	Radius of Nucleus	Nuclear Volume	Radius of Cell	Cell Volume	Chromosome Length	Chromosome Width	Volume of Single Chromosome
2 cell.....	12.584μ	8347.2μ^3	28.8μ	100,000.μ^3 (99,450.)μ^3	8.53μ	1.5μ	19.17μ^3
4 cell.....	7.034μ	1598.0μ^3	22.7μ	43,000.μ^3 (49,725.)μ^3	6.12μ	1.3μ	10.343μ^3
8 cell.....	6.16μ	956.8μ^3	17.47μ	22,343.^{3} (24,862.)μ^3	5.78μ	1.2μ	7.906μ^3
16 cell.....	5.17μ	578.8μ^3	13.074μ	9,361.6μ^3 (12,432.)μ^3	5.147μ	1.0μ	5.147μ^3
32 cell.....	4.92μ	500.8μ^3	10.47μ	4,814.μ^3 (6,216.)μ^3	4.46μ	1.0μ	4.46μ^3
64–132 cell....	3.88μ	245.14μ^3	7.309μ	1,633.5μ^3 (1,556.3108)μ^3	4.27μ	0.9μ	3.458μ^3
Blastula I....	2.92μ	103.23μ^3	5.66μ	759.5μ^3 (778.)μ^3	3.76μ	0.8μ	2.406μ^3
Blastula II....	2.232μ	46.58μ^3	3.586μ	193.3μ^3 (194.)μ^3	3.2μ	0.8μ	2.083μ^3
Gastrula I...	2.05μ	36.07μ^3	3.321μ	152.6μ^3	2.28μ	0.8μ	1.459μ^3
Gastrula II...	1.81μ	25.03μ^3	3.0758μ	121.8μ^3	2.00μ	0.5μ	0.5μ^3
Pluteus.....	1.62μ	17.81μ^3	2.44μ	53.0μ^3	1.00μ	0.45μ	0.2042μ^3

claimed by Erdmann, the total amount of chromatin up to the 64-cell stage increases in amount and in the blastula stage is still greater. On the other hand, Baltzer finds that the chromosomes of the blastula stage are much smaller than those of the first cleavage. Erdmann compares the relation of the total volume of the chromatin to the volume of the blastomere, the relation of the nuclear volume to the volume of the cell, and of the chromatin volume to that of the nuclear volume; (Table XVII).

From this table it appears that the chromatin in the pluteus is seven times greater in proportion to the protoplasm of its cell. Up to the blastula stage the chromatin volume increases in each cell in relation to the protoplasm. From this time onward there is a small change to the disadvantage of the chromatin. There is a decrease throughout in the proportion of cell volume to nuclear volume, since at each division each cell is reduced to half.

It follows that, while there is relatively more chromatin at the end of cleavage than at the beginning, there is not nearly

so great an amount of chromatin as there would be had the chromosomes remained the same size. Whether the cessation of rapid cell-division is due to the exhaustion of the materials out of which the chromatin is made, cannot be stated, however plausible such an assumption may appear. There may be other factors that inhibit cell-division, and also the growth of the chromatin.

TABLE XVII

NORMAL CULTURE

Stage	Cell Volume : Chrom. Volume	Cell Volume : Nuclear Volume	Nuclear Volume : Chrom. Volume
2 cell	326 : 1	12 : 1	27.2 : 1
4 cell	269 : 1	26.9 : 1	10.1 : 1
8 cell	176 : 1	23.4 : 1	7.4 : 1
16 cell	113 : 1	16.8 : 1	7.1 : 1
32 cell	69 : 1	9.6 : 1	7.2 : 1
64–132 cell	29 : 1	6.7 : 1	4.4 : 1
Blastula I	19 : 1	7.4 : 1	2.6 : 1
Blastula II	5.6 : 1	4.1 : 1	1.3 : 1
Gastrula I	6.7 : 1	4.2 : 1	1.5 : 1
Gastrula II	15.2 : 1	4.9 : 1	3.1 : 1
Pluteus	19.2 : 1	3.7 : 1	5.4 : 1

If the size of the genes be constant, their total number must be thousands of times greater in the full grown animal than in the fertilized egg; but the amount of protoplasm is also many times greater. The really important fact to be kept in mind is that the observational evidence goes to show that during development every cell receives the entire complex of hereditary units. Conversely, there is no evidence in favor of the view that there is any change in the genetic constitution of the chromosomes as development proceeds. It may be claimed, of course, that the genes are so small that even were they to some extent sorted out during cleavage, the process would escape detection, for they are beyond microscopic vision. This must be conceded, but there is evidence from other sources that indicates that no such process takes place.

The relation of nuclear size to volume of cytoplasm has been examined in fragments of sea-urchin's eggs, both diploid and haploid. Fragments of the eggs that are even smaller than the

micromeres of the 16-cell stage may be fertilized. There are no accurate measurements of the size of the nuclei that appear in these fragments, but the nuclei are reported to be much smaller than the nuclei of corresponding stages of the whole egg. Since these fragments divide by mitotic division, it follows that the division of the nucleus is not dependent on its absolute size in order to become resolved into a mitotic figure.

During the cleavage stages of the diploid fragments, the resting nuclei are smaller than are those of corresponding stages of the whole egg (Morgan '93, '95). Comparisons between later stages at the time of gastrulation, etc., are difficult to make, since, in general, fewer cells, hence relatively more cytoplasm per cell, are present in the smaller larvae. That whole larvae, whether entirely haploid, or regionally haploid, have smaller nuclei than normal diploid larvae has often been reported (Boveri, Baltzer, Herbst). The presence of half the number of chromosomes in cells with the normal full amount of cytoplasm offers a somewhat different problem from that discussed above.

Conklin ('12) has made measurements of the chromosomes of some of the blastomeres of Crepidula plana. The number of chromosomes is so large (about 60) that it is not possible to measure them all, or even to follow the history of individual chromosomes, nevertheless the results suffice to show the general trend of change in their sizes. He finds that while the chromosomes are smaller in the later cleavage cells than at the beginning, nevertheless the decrease is slight in comparison with the decrease in size of the nuclei. Despite the reduction in size the actual amount of chromatin material must be vastly greater after several thousand cells have been formed than it was at the beginning of cleavage.

A few chromosomes from the spindle of the two-celled stage are shown in Fig. 54*a*, and a few others from the first division of the first quartet cells (1a–1d) are shown in Fig. 54*b*, both magnified 2000 diameters. The former from the 2 larger cells are larger (5.2 cubic μ) than those from the smaller micromeres (2.6 cubic μ), but the differences are not so great as the differences in the volumes of the nuclei from which they came. Thus while the volumes of the nuclei, from which the chromosomes came, are about as 5 to 1 the volumes of the individual chromosomes are as 2 to 1.

A further attempt to measure the changes in chromatin in Crepidula plana was made by measuring the sizes of the metaphase plate in successive stages. This method, while not so accurate in some respects, should give sufficiently definite information to be of value. The dimensions of the chromosomal plate from the 2-cell to the 32-cell stage are given in Table XVIII. On an average there is an increase of 8 per cent for each of the three divisions involved. The total chromosomal mass increases in volume 284 per cent, but the chromosomes grow smaller as cleavage advances. Whenever there are both large and small cells dividing in the same generation, the smaller cells have smaller chromosomal

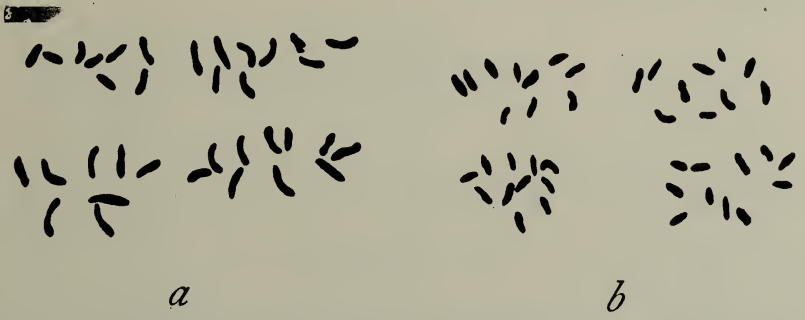


FIG. 54.—*a*, chromosomes from the second cleavage-spindles of Crepidula; *b*, chromosomes from the first four micromeres of Crepidula. (After Conklin.)

plates (and smaller chromosomes) than the larger cells. The results are in harmony with those of Erdmann and of Godlewski.

It is much easier to measure the size of the resting nuclei in different blastomeres, and Conklin has made a study of the sizes of the nuclei in relation to the sizes of the cells of Crepidula from the egg to the seventh cleavage. Only a few of the results can be taken up here. The nucleus changes in size during the resting stage of the cell. Its increase in size (growth) is more rapid toward the end of the resting period than at any other time. Its maximum size is reached just before the nuclear wall disappears. The results are given in Table XIX. It is clear that while larger blastomeres have larger nuclei than smaller ones, the relation between nuclear volume and cell volume is not constant. The nuclear-plasma relation varies from 1 to 14.5 to 1 to 0.37. Even in cells that have little yolk it varies from 1 to 14.5 to 1 to 8.7.

It has sometimes been stated that the nuclear-plasma relation of embryonic cells is different from that of tissue cells. Minot ('90, '95, '08) tried to show that differentiation and senescence come about through an increase in the protoplasm as compared with the nucleus. This conclusion is not borne out in the case of Crepidula. As shown in Table XX, where the nuclear and

TABLE XVIII

MINIMUM NUCLEAR SIZE AND CELL SIZE IN THE BLASTOMERES OF CREPIDULA PLANA; (NUCLEAR PLATE MEASURED IN THE LATE ANAPHASE; CELL DIAMETER IN EARLY TELOPHASE)

Stage Blastomeres	Diameter of Cell, μ	Diameter of Protoplasm Including Nucleus, μ	Dimensions of Nuclear Plate, μ	Volume of Nuclear Plate, Cubic μ	Volume of Protoplasm Less Volume of Nucleus, Cubic μ	Kernplasma-Relation
2 cells A, B, C, D.....	105	Ca. 46	9×3	190.5	30,504	1 : 160
4 cells A, B, C, D.....	78	Ca. 40	8×3	150.6	30,695	1 : 203.8
8 cells 1A–1D.....	75	Ca. 36	6×3	84.6	24,166	1 : 285.6
8 cells 1a–1d.....	27	27	6×3	84.6	10,235	1 : 120
12 cells 2A–2D.....	72	Ca. 30	6×3	84.6	13,955	1 : 165
12 cells 2a–2d.....	30	30	6×3	84.6	13,955	1 : 165
16 cells $1a^1$–$1d^1$.....	30	30	5×3	58.8	13,955	1 : 237
16 cells $1a^2$–$1d^2$.....	15	15	5×3	58.8	1,708	1 : 29
20 cells 3A–3D.....	72	Ca. 15	4×3	37.5	1,729	1 : 46
20 cells 3a–3d.....	25	25	4×3	37.5	8,087.5	1 : 215.6
24 cells $2a^1$–$2d^1$.....	24	24	4×3	37.5	7,200	1 : 195
25 cells 4D.....	60	.....	5×3	58.8		
25 cells 4d.....	30	.....	5×3	58.8		
29 cells $2a^2$–$2d^2$.....	24	24	4×3	37.5	7,200	1 : 195
29 cells $1a^{1.1}$–$1d^{1.1}$.....	15	15	4×3	37.5	1,729.5	1 : 46
29 cells $1a^{1.2}$–$1d^{1.2}$.....	24	24	4×3	37.5	7,200	1 : 195
32 cells 4A–4C.....	.....	.....	4×3	37.5		
32 cells 4a–4c.....	.....	.....	4×3	37.5		

cell sizes of some of the tissue cells are shown, there is no marked increase of protoplasm over nucleus as compared with the blastomeres. Throughout the cleavage with the exception of the cells 3A and 3D and 4A and 4D the average "Kernplasma-Relation" for nuclei and cells of mean size is about 1 : 15; for nuclei and cells of maximum size about 1 : 6; the average ratio in adult tissue cells is about 1 : 10.5.

TABLE XIX

MAXIMUM NUCLEAR SIZE AND CELL SIZE IN THE BLASTOMERES OF CREPIDULA PLANA; (MEASURED JUST BEFORE NUCLEAR MEMBRANE DISSOLVES)

Stage Blastomeres	Diameter of Cell, μ	Volume of Cell, Cubic μ	Diameter of Protoplasm Including Nucleus, μ	Diameter of Nucleus, μ	Volume of Nucleus, Cubic μ	Volume of Protoplasm Less Volume of Nucleus, Cubic μ	Kernplasma-Relation
Before maturation	150	1,755,000	Ca. 64*	42	32,409	97,131	1 : 3
Before first cleavage	142	1,488,910	Ca. 65*	*♀30+♂24 =34.5	21,375	121,430	1 : 5.6
AB, CD, before second cleavage	106	619,329	Ca. 51*	24	7,238	61,741	1 : 8.5
A, B, C, D, before third cleavage	82	286,712	Ca. 44*	22	5,775	38,570	1 : 6.6
1A–1D, before fourth cleavage	81	276,350	Ca. 40*	21	4,849	28,431	1 : 5.8
1a–1d, before division	.....	.....	30	14	1,437	12,603	1 : 8.7
2A–2D, before fifth cleavage	80	266,240	Ca. 36	18	3,055	21,196	1 : 7
2a–2d, before division	.....	.....	36	15	1,767	22,484	1 : 12.7
$1a^1$–$1d^1$, before division	.....	.....	30	12	905	13,135	1 : 14.5
$1a^2$–$1d^2$, before division	.....	.....	15	7	180	1,587	1 : 8.8
3D, before sixth cleavage	76	228,288	Ca. 30	16	2,145	11,895	1 : 5.5
3A–3C, before sixth cleavage	76	228,288	Ca. 22	16	2,145	3,430	1 : 1.6
3a–3d, before division	.....	.....	33	14	1,437	19,250	1 : 13.3
$2a^1$–$2d^1$, before division	.....	.....	30	14	1,437	12,603	1 : 8.7
$2a^2$–$2d^2$, before division	.....	.....	30	14	1,437	12,603	1 : 8.7
4d, before seventh cleavage	38	28,533	Ca. 22	11	697	4,878	1 : 7
4A–4D, before seventh cleavage	60	112,320	Ca. 20	18	3,055	1,134	1 : 0.37
4a–4c, before seventh cleavage	42	32,409	Ca. 14	12	905	532	1 : 0.58

* After yolk has been centrifuged out of egg. In normal egg, yolk and protoplasm are not well segregated at this stage.

Interesting results bearing on nuclear size have been obtained by centrifuging the eggs. When one cell contains much protoplasm and its sister cell only yolk, the size of the nucleus that appears in the former may be many times larger than that in the latter. It is evident that the imbibition of fluid, to form the

cell-sap of the nucleus and determine thereby its size to a large extent, depends on the amount of the protoplasm in the cell. The protoplasm, here, may mean no more than the amount of watery substances present that are taken up by the reforming nuclei as nuclear sap. Whether the size of the chromosomes is also dependent on the same factors in such cases has not been shown.

TABLE XX

Cell Size and Nuclear Size in Tissue Cells of Sexually Mature Individuals of Crepidula Plana

Tissue Cells	Dimensions of Cell, μ	Diameter of Nucleus, μ	Volume of Nucleus, Cubic μ	Volume of Cell Less Volume of Nucleus, Cubic μ	Kern-plasma-Relation
Intestinal epithelium	11×11×12	6	113	1,339	1 : 11.8
Gastric epithelium	10×10×36	8	68	3,332	1 : 12.4
Liver duct epithelium	10×10×18	6	113	1,628	1 : 14.4
Liver cells (filled with secretion products)	15×15×45	6*	113	10,012	1 : 88.6
Liver cells (without secretion products)	14×14×30	9	382	5,498	1 : 14.4
Kidney cells (containing secretion products)	15×15×15	6	113	3,262	1 : 28.8
Ectodermal epithelium (near anus)	5× 5×15	4	33	342	1 : 10.3
Gill chamber epithelium	6× 6×12	4	33	405	1 : 12.2
Gill filament epithelium	7× 7× 9	4	33	408	1 : 12.3
Epithelium from foot	6× 6×15	5	65.4	474.6	1 : 7.1
Ganglion cell (large)	17×17×23	12	905	5,724	1 : 6.3
Ganglion cell (large)	10×10×20	9	382	1,618	1 : 4.2
Oöcytes I (before yolk formation)	$12\frac{1}{2}$	7	180	836	1 : 4.6
Oöcytes I (before yolk formation)	$11\frac{1}{2}$	7	180	791	1 : 3.4
Oöcytes I (before yolk formation)	10	6	113	407	1 : 3.6
Oöcytes I (before yolk formation)	8	5	65.4	203	1 : 3.1
Oöcytes I (before yolk formation)	$6\frac{1}{2}$	4	33	111	1 : 3.3

* Nucleus shrunken and very irregular in shape.

How the chromosomes, or rather the genes that are their essential constituents, bring about changes in the cytoplasm of the cells is unknown. There is at least no evidence opposed to the view that they do this by chemical processes. It is impossible to suppose that the genes themselves could be thrown off and could pass through the nuclear membrane into the cell. If they did so they would lose their linear order, which, the genetic evidence demon-

strates for the germ-track at least, is maintained. It is not so difficult to imagine that through their activities chemical substances are produced that find their way into the cytoplasm either at the time when the nuclear wall is dissolved and nuclear sap set free, or possibly by diffusion through the nuclear walls.

The arrangement of the chromosomes in a cell plate at the metaphase of each mitosis has often been commented upon. In some cases the smaller chromosomes are more often found in the middle of the plate; in other cases the members of a pair are often found near together, but the fact that frequent exceptions to these rules have been observed appears to indicate that the arrangement is of no great significance. It appears in several instances that the relative position of the chromosomes is largely

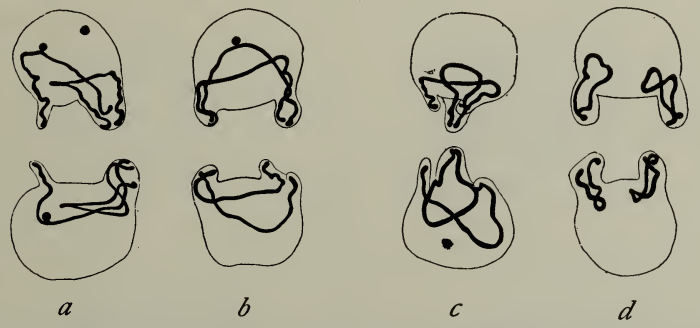


FIG. 55.—Daughter blastomeres of Ascaris in which the sister chromosomes are about to appear at the next division. The sister chromosomes are mirror figures of each other. (After Boveri.)

the result of the position they occupied when, at the end of the preceding division, they went into a resting stage, as shown by Boveri's observations on the eggs of Ascaris (Fig. 55*a-d*). There must, however, be a probable position that a given chromosome will assume aside from this relation, since the smaller ones frequently lie in the center of the group and the larger ones at the periphery. It may only mean that this is the more stable position of the chromosome vesicles, which form in the anaphase, provided at this time they are free to move with respect to each other. Such an arrangement is not unlike the position taken by smaller and larger oil drops in a confined space, as Roux and others have pointed out. In the latter cases the relative position of the drops is explicable on their surface tension relations.

R. S. Lillie ('03, '05, '11) has pointed out the similarity in the arrangement of the chromosomes at the metaphase and the positions taken by floating magnetic needles or threads bearing electrified beads. Similar configurations might also result if around each chromosome there is a region of relatively greater density than that of the protoplasm in which they lie.

Individuality of Chromosomes

The constancy in the number of chromosomes in the cells of each species and each sex, the relative constancy in size of the members of each group, and the persistence of the size relations of the chromosomes when they are ready for division furnish strong arguments for the persistence of the chromosomes. The obvious conclusion is that this constancy is due to continuity of individual chromosomes from one cell-generation to the next. There is, however, an alternative point of view which has been maintained by several writers, especially by Oscar Hertwig, namely, that the constancy in number is a peculiarity of the cells of each species. According to this interpretation, the chromatin reforms at the beginning of each division out of the chromatin of the resting nucleus in definite forms as crystalloid substances do from a super-saturated solution. Which alternative is the more probable will depend of course, on the evidence that is forthcoming. The direct evidence in favor of the first view is rather limited, but the indirect is quite strong. This evidence may be first considered.

Boveri ('02, '08) observed that when, after the first or second division of the egg of Ascaris, the nuclear threads of the anaphase change into vesicular-like structures, the shape of the nucleus, especially on the sides of the daughter nuclei facing each other, is often irregular in outline conforming to the position of the ends of the chromosomes (Fig. 55*a-d*). There was often a marked similarity in the irregular outline of the two daughter nuclei, because their chromosomes had the same shape at the end of the anaphase. He found that when the chromosomes reappear preparatory to the next division the ends of the threads in sister cells are mirror figures of each other. The most probable conclusion is that the chromosomes, in spite of their vesiculation, have remained in nearly a constant position in the resting nucleus,

or, in other words, that they have not been broken up or dissolved and reformed. As far as this evidence goes it stands for continuity, i.e. it means that the chromosomes in a resting stage retain their form and position.

Wenrich ('16) and others have observed in the sperm-cells of

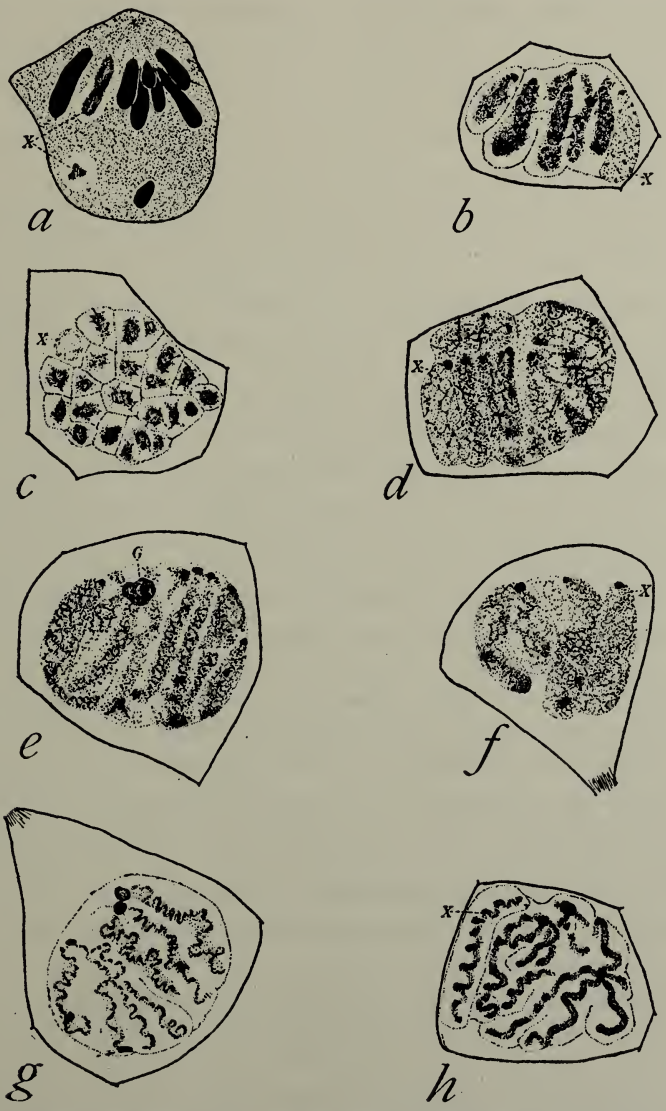


FIG. 56.—*a*, spermatogonial cell in the last phase of division; *b*, *c*, *d* in the following resting stages; and *e* and *f*, in the preparatory stages for the next division. (After Wenrich.)

grasshoppers that each daughter chromosome at the end of division forms a separate vesicle (Fig. 56). These vesicles, that collectively make up the new nucleus, can be followed almost through the resting stages. Vesicles also reappear when the nucleus prepares for the next division. It seems most probable that the vesicles, each with its contained chromosome, remain essentially intact throughout the resting period. It must be added, however, that in most cases there is practically no evidence that the chromosomes remain individually enclosed in vesicles. The special case of the sperm-cells may be due to the relative rapidity of the successive divisions at this time.

The best experimental evidence in favor of the continuity of the chromosomes is derived from genetics. The chromosomes have been shown to be made up of genes arranged in linear order. Each gene occupies a definite place with respect to the other genes in the same chromosome. This order remains the same through successive divisions. It is inconceivable that the thousands of genes could dissolve in the protoplasm after each division and come together when the chromosomes reform. It has been shown moreover, by genetic work, that during the maturation of the germ-cells there may be an interchange between homologous chromosomes. The interchange involves like-parts, and the order of the gene remains the same as before in each chromosome.

The evidence for continuity does not, of course, preclude the possibility that the genes may at times break apart. When this happens it appears that they remain as separate parts, and do not subsequently reunite. There is also other genetic evidence that two chromosomes may unite end-to-end to form a new stable unit. And there is evidence also that whole sections of chromosomes may be reversed in the sense that their genes come to lie in reversed order. All these possibilities produce interesting genetic complications, but as they are rare they do not conflict with the view that the chromosomes are relatively stable structures. In fact, the exceptions go far toward supporting the rule.

Boveri's Evidence that a Complete Set of Chromosomes is Necessary for Normal Development

It had been shown that dispermic eggs of the sea-urchin (eggs that divide in a threefold or fourfold cleavage pattern) do not

produce normal embryos (Driesch '92; Morgan '95), but whether this was due to the method of division of the cytoplasm, or to the irregular distribution of the chromosomes was not apparent. If, at the time of cleavage, cytoplasmic changes (movements) take place that are important for the subsequent events, it is conceivable that these variations of the cleavage pattern might in themselves lead to abnormal development. On the other hand, if the chromosomes have an irregular distribution this result might be held responsible for the later abnormalities.

It was also known from still earlier work on dispermic eggs of the sea-urchin (Hertwig, O. and R., '87) that a three-pole (triaster) or four-pole (tetraster) mitotic figure develops (each sperm supplying two centrosomes) and that the chromosomes are distributed irregularly to the poles (Fig. 8). Consequently each blastomere may receive different numbers of chromosomes. If this irregularity in chromosome distribution affects the subsequent stages of development, abnormalities might be expected; but, on the other hand, since the cytoplasm of the egg has been formed under the influence of a normal diploid group of chromosomes this influence might be expected to carry on throughout the early stages at least, and give normal development in spite of the secondary distribution of the chromosomes. In fact, the cleavage of dispermic eggs is normal except as to the pattern, and actively swimming blastulae develop from these dispermic eggs. Gastrulation, however, may often be abnormal, and abnormal plutei result, if the development reaches this stage.

Boveri ('02) took advantage of the irregular distribution of the chromosomes in the dispermic eggs to study the question as to whether the abnormality of the development of each egg is due to the chromosome distribution. It was known that when the blastomeres of the normal two- or four-cell stage are shaken apart, each may develop into a whole embryo. In this case each blastomere has the normal or diploid number of chromosomes. Boveri carried out the same procedure with the threefold and fourfold types of blastomeres of dispermic eggs. Here each may receive a different number of chromosomes. On chance alone a few of them might be expected to contain one set of chromosomes at least, as well as other chromosomes. The chance for one set would seem to be slightly greater when the triploid group of chromosomes is separated into three parts than when, as in the

fourfold type, the group is separated into four. There is, however, a further implication that was not realized at the time. Normal development would not be expected to occur (except perhaps for the earliest stages as explained above) even when one complement of chromosomes (i.e., when one of each kind) is present if other chromosomes were also present, for these, as the genetic evidence has more recently shown, might upset the balance of the genes that is essential for normal development. This point will come up again after Boveri's results have been examined.

The haploid number of chromosomes in the reduced egg and sperm in the species Boveri used is eighteen. The normal diploid eggs have thirty-six. The triploid egg (that may divide at once in three or four parts, according to whether three or all four of the centers take part in the division) will have three times eighteen, or fifty-four chromosomes. Each divides into two daughter chromosomes, and one hundred and eight chromosomes are ready to move toward the three poles. The numerical problem is how often, on chance alone, will any one of the three or four poles be likely to get at least one full set; but the situation is really more complicated than this, if, as seems probable, the daughter chromosomes tend to move away from each other; and furthermore, whether from their position in groups of threes there is a greater chance of a haploid set passing to one center than when these relations are not involved. There are too many unknown conditions present to make such calculations of much value. Boveri argued that, when the one hundred and eight chromosomes are separated into three groups, it is more probable that each blastomere will receive one haploid set at least, than when separation takes place into four groups (Fig. 57). It is from this point of view that Boveri attempted to analyze his results.

On this assumption, then, the $\frac{1}{3}$ isolated blastomere might be expected more often to contain one complement of chromosomes and produce normal plutei than the $\frac{1}{4}$ blastomere from dispermic eggs. Boveri's observations showed that the $\frac{1}{3}$ isolated blastomeres do more often develop as far as the pluteus stage than do the $\frac{1}{4}$ isolated blastomeres. From which he concluded that one set of chromosomes at least is essential for division. While this conclusion is consistent with recent genetic evidence, the argument that Boveri used is open to the criticism already mentioned that,

even while one set (haploid) may be necessary, the presence of other chromosomes in the group may so seriously affect the outcome that the value of the deduction is greatly impaired.

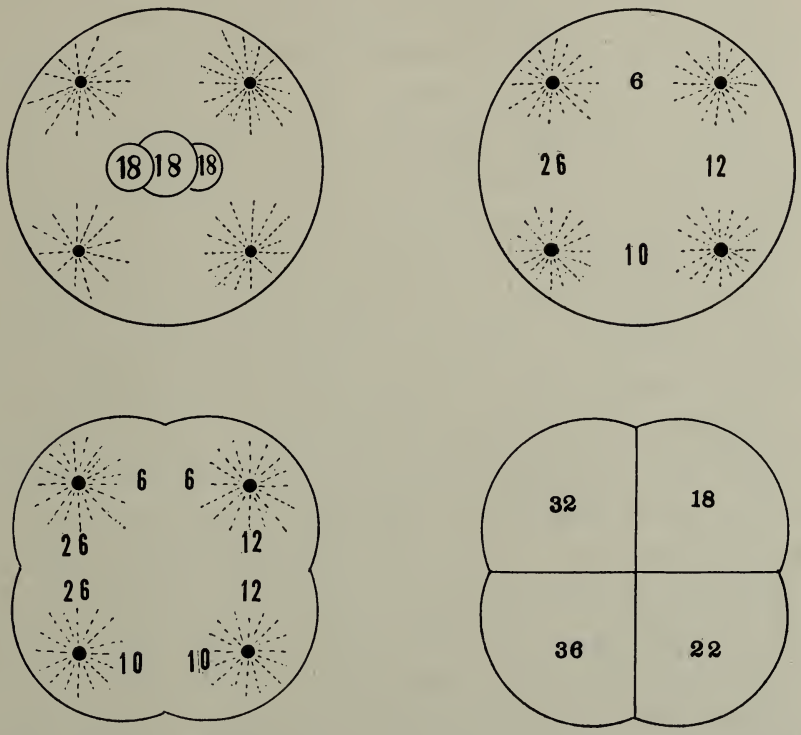


Fig. 57.—Diagram of dispermic egg of sea-urchin with $3 \times 18 = 54$ chromosomes and four asters (above to left). The other figures show one of the many possible distributions of the chromosomes to the four resulting cells. (After Boveri.)

Genetic Evidence Relating to Chromosomes

Embryological work has given no information as to the make-up of the chromosomes themselves. So far as this evidence goes, each chromosome might be like every other one, and each might be composed of identical material throughout. On the other hand, genetics has, by analytical methods, succeeded in penetrating inside the living cells and in discovering there the intimate constitution of the individual chromosomes. It would be out of place here to go into the methods by which the results have been obtained (in the main by a study of crossing-over), but since the conclusions

are of prime importance for an understanding of the relation of the chromosomes to the cytoplasm of the developing egg, they may be briefly stated.

The evidence has shown that each chromosome is composed of a large number of elements, or genes, arranged in linear order. The genes are constant in number and in arrangement, i.e., in their sequence in the chromosomes. The evidence indicates that the genes are different from each other, not only in the same chromosome, but also in different chromosomes. It is true the last statement is an inference based on the fact that when any one of the genes changes, it is instrumental in producing a different end-result (the product of all the genes) from that produced when any other gene changes. The inference is further supported by the fact that the new gene has a different relation to the original gene from which it came than it has towards any other gene.

The specific action of each gene on the end-product of all the genes does not mean that each gene is a determiner for a particular character, although it may have a more direct relation to one or to several, or even to many characters, than to the rest of the individual. This relation may be expressed in the following way: The characters of the individual are to be regarded as the end-product of the activity of all the genes. If one gene changes, the end-product will be, to some degree, affected. If the resulting change in the character is very great, it may lead to the death of the individual (lethal factors), or may weaken the individual or make it monstrous or abnormal to some degree. If, however, the change is great, but affects some less important part of the individual, as when the red eyes of Drosophila become white, or the brown eyes of man become blue, the individual may be viable. On the other hand, there is abundant evidence showing that many of the changes in the genes may affect the individual to a very slight extent. The resulting effect may be so small that it is a matter of no consequence in the life of the individual. It is also theoretically possible that some of these lesser changes may be of advantage to the individual. This, however, is less to be expected, since each organism is already highly adapted to a very complex environment, and amongst the many possible random changes there may be only a limited number that make the new individual better off than the original type. It is, then, not to be expected that amongst the frequent mutational changes

that are observed there will often be found improvements. Nevertheless, the whole history of our domesticated animals and cultivated plants shows that very great changes may be brought about by the accumulation of small mutational alterations, and some at least of these changes are of benefit to the individual, if only because it leads to their survival under the environment supplied by man.

The multiple effects of a change in any one gene calls for emphasis in this connection. The genetic evidence has abundantly shown that when a single gene is changed, the end-product may be affected in many ways. We select the most apparent, and for our purposes, most convenient change and identify it as the immediate product of the new gene. But as is well understood, this is only a useful method in studying the inheritance of the genes. It is perfectly well known that, besides certain major effects, there are many accompanying effects also present involving all parts of the body, and this result is entirely consistent with the theory that all the parts are the products of all the genes. A particular change in a gene may visibly affect certain regions more strikingly than it affects others, and these are the characters that we use, *as a rule,* for diagnostic purposes.

Most of the characters selected for study are structural changes, because these are visible and more easily followed, but there is ample evidence to show that physiological effects are produced, many of which may be accompaniments of the structural changes; and, *vice versa,* physiological effects may carry in their train structural changes. The fact that more of the diagnostic characters studied by geneticists relate to superficial characters has misled a few embryologists into supposing that changes in the chromosomes are confined to superficial differences, while the more "fundamental" characters are cytoplasmic in origin. Nothing could be further from the established evidence than this conclusion; for, while it is true that many of the inherited characters studied by geneticists do relate to "superficial" characters, the explanation is to be found in the fact that it is this sort of character—because it is trivial for the life of the individual—that is better suited to follow through successive generations than are those characters that cripple, or kill, or sterilize the individual carrying them. Moreover, geneticists are fully cognizant of many kinds of characters that make the individual more fertile, or in-

volve the fundamental symmetries of the structure, or effect profound physiological differences, both advantageous and harmful.

One of the most important questions for embryology relating to the activity of the genes cannot be answered at present. Whether all the genes are active all the time, or whether some of them are more active at certain stages of development than are others, are questions of profound interest. Either alternative can be made compatible with what we know at present concerning development—little as this may be—but until we can get information on this point, it will not be possible to offer a satisfactory theory of the way in which the genes affect development, and we must wait for further advances either in embryology or genetics. Neither can we reach a decision, at present, as to whether the genes produce their principal effects during the resting stage of the nucleus, or only when the nuclear wall dissolves, and the nuclear plasm is set free to mix with the cytoplasm. If the substance produced directly by the genes, or the substance produced in contact with the genes, is diffusible through the nuclear wall, it is quite possible that the genes are acting throughout the "resting-stage,"—a term that relates to the form of the nucleus, and not necessarily to its functioning. If the substances that affect developmental changes, as apart from those that concern the physiology of the differentiated cell, can be set free from the nucleus only when its walls are dissolved, it is possible that the periods of nuclear and cell-divisions are the times at which these effects are produced.

It has been suggested a number of times that the genes themselves are enzymes and that their action is brought about as catalyzers of the cytoplasmic substances. There are two quite distinct questions here. The genes may produce directly, or be indirectly contributory to, the formation of enzymes or other catalytic substances. If, as there is indirect but significant evidence to show, such changes are influential in development, it may well be that the genes are instrumental in one way or another in producing catalytic materials. On the other hand, there is no sufficient evidence to show that the genes themselves are enzymes. The best argument that they are not is found in the behavior of extracellular enzymes and other catalysts, which do not increase in amount as they bring about certain results, but, on the contrary, remain constant in amount or may even decrease during

the catalytic reaction. During development, especially during the early cleavages, the amount of chromatin steadily increases in amount, giving an exponential curve resembling the first half of a curve of a monocatalytic reaction. It is more than hazardous to conclude from this that the increase in the chromatin is itself evidence of a monocatalytic process, for many kinds of increases that have nothing to do with such a process, will give the same form of curve. It is true that if the chromosomes are directly or indirectly concerned with the making of enzymes, then the larger the number of chromosomes present, other things being equal, the more enzymes might be made, but it is a far cry from this interpretation to the assertion that the chromosomes are themselves strings of enzymes.

If, then, as the only evidence we have today indicates, the chromosomes are made up of, or contain a very large number of very small particles, the genes, that are all different from each other (two of each being present in each homozygous individual corresponding to the two chromosomes of each kind), it does not follow from a chemical standpoint that the genes in any individual may actually be very different from each other. For all we know to the contrary they may all be built upon a common radicle, and these radicles may be somewhat similar or even identical in species that closely resemble each other (historically related individuals having had a common descent). The difference between them may, in a chemical sense, be quite trivial (side chains). It remains for future work to discover how far and in what respects the genes actually differ from one another.

CHAPTER XIII

MECHANICS OF ORGAN-FORMATION

As a result of cleavage the egg becomes divided into a number of cells. In nearly all cases the cells of one hemisphere are smaller than those of the other, but there is no sharp line of demarkation between the smaller and the larger cells. The early cleavages start at the surface, and cut into the egg in a radial direction. At a very early cleavage stage—frequently at the four-cell stage—a cavity filled with fluid appears in the interior, the segmentation cavity or blastocoel. As the cleavage proceeds the blastocoel slowly enlarges and in many eggs comes to occupy the whole interior of the embryo. In some cases the surface of the embryo may consist at one stage (blastula) of a single layer of cells, as in Amphioxus (Fig. 58*a*) and starfish; the cells of one hemisphere being smaller than those of the other. In other cases as in the frog, the blastula wall consists of more than one layer of cells. The double layer has been brought about by cleavage planes appearing either parallel to the surface, or obliquely to it with subsequent rearrangements.

The segmentation cavity is filled with a fluid that is coagulated by the usual killing reagents. It probably contains a small amount of albuminous material that is secreted by or squeezed out of the surrounding cells. Probably water also infiltrates into the interior either between the blastomeres or through the cells. In several cases the volume of the whole embryo in the late segmentation stages is not very much greater than that of the egg, as in the sea-urchin where the cells occupy only the outer shell and the interior is filled by the large segmentation cavity. Consequently it appears that the mass of protoplasm (now in the surface layer) has actually decreased and it is probable that the loss is due to fluid poured into the blastocoel.

Embryo formation begins in typical cases by a process of inturning of the cells of that hemisphere that contains more of

the yolk (Fig. 58*b*, *c*). This process, called invagination or gastrulation, results in a two-layered embryo, the gastrula. The inturned hemisphere is called the archenteron. The simplest cases are those where a single layer of cells is present over the surface of the blastula wall and about half of them are invaginated as in Amphioxus (Fig. 58*a*, *b*, *c*), sea-urchin, and in the ascidian

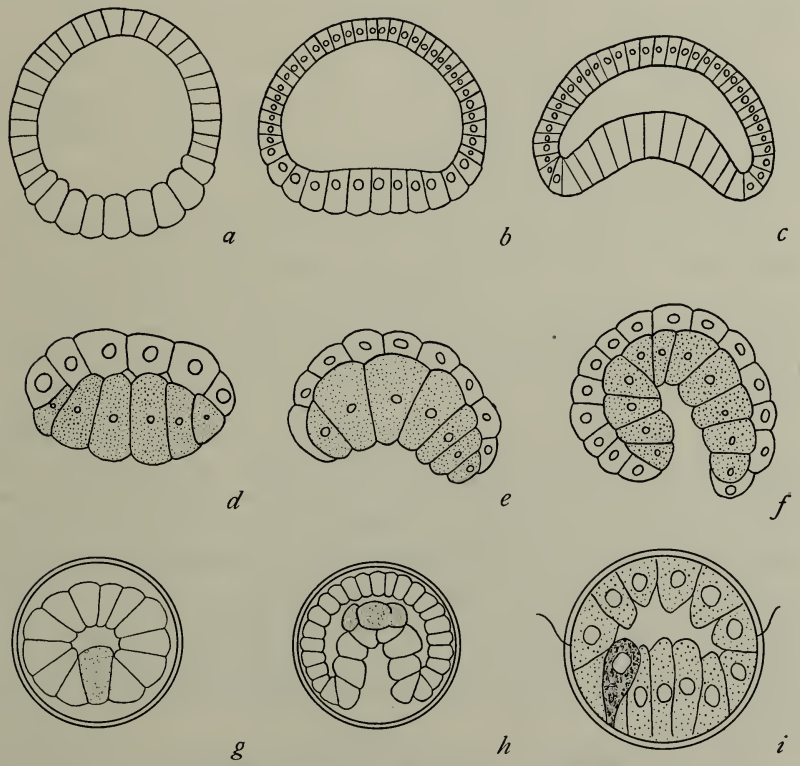


Fig. 58.—*a*, *b*, *c*, gastrulation of Amphioxus; *d*, *e*, *f*, gastrulation of Clavellina. *g*, *h*, gastrulation of Lucifer; *i*, gastrulation of Eupomatus. (After Rhumbler.)

(Fig. 58*d*, *e*, *f*); but in other cases the number of invaginated cells may be much smaller, even no more than one or two cells. From such simple processes, other methods of gastrulation are represented as arising. This interpretation is a tradition of the quasi-historical method of handling embryological problems that still prevails in all descriptive embryology. On the other hand some of the simpler methods, such as that in Amphioxus, may well be secondary simplifications. It is not necessary here

to assume that any one method of gastrulation is to be regarded as the typical process from which the others have been derived. From the point of view of the mechanics of development it is a matter of no special moment what the historical sequence has been since we are concerned in each case with what is going on at the time. Those cases, that show in the clearest way what changes take place, seem to offer the most promising opportunity for analysis in terms of physical processes.

Although embryologists have from time to time hazarded guesses as to what causes the cells in one hemisphere to invaginate, there are relatively few writers who have attempted seriously to work out the mechanism involved.

One of the earliest attempts to analyse the gastrulation process was that of Ludwig Rhumbler ('02). He pointed out that invagination would result if some external force pushed the wall in at some point, or if some internal agent sucked the wall into the interior, or if the wall were forced in by increased tangential pressure within the wall itself. These three possibilities he further considered.

Under normal conditions of development there is no external agent that can be appealed to that is capable of pushing in the wall unless it were a higher osmotic pressure. But the process takes place in an unchanging medium, and even if it were due to such an agent, it is the thin wall of the "animal" half that would be expected to yield first. The membrane that surrounds the blastula is, as a rule, not in contact with the surface of the embryo, hence the membrane could not be an active agent in the process. Moreover, many embryos gastrulate after they have left the egg shell.

The wall could be sucked in only as a result of a decrease in the blastocoel fluid. Such a decrease actually may occur during gastrulation. Hatchek suggested that the blastocoel fluid is absorbed by the endoderm cells and that in consequence this region invaginates. But the endoderm cells may not increase in size at this time although they change their shape. Even if they should increase in size slightly, they do not do so sufficiently to account for the disappearance of all of the interior fluid. Furthermore, it is the thinner wall, and not the thicker one, that would turn in if a decrease in internal pressure took place, and this regardless of what cells absorbed the fluid.

If pressure, tangential in direction, should arise within the wall of the blastula due to an increase in the number of cells followed by growth to the original size, the wall ought to bulge out at some point rather than turn in, since the cells of the wall are, in section, wedges, or pyramidal in shape with the broad base on the outside.

As the cells of one hemisphere, or part of it, are turned in, the pressure between the cells of the other hemisphere will be lowered, since the line of cells, so to speak, becomes longer, and the smaller cells will be expected to expand, and this, in fact, occurs. It is the observed expansion of the smaller ectoderm cells that has led several embryologists to suppose that the pressure of these cells on each other is the physical force that causes the "weaker" cells of the yolk hemisphere to turn in; but, as Rhumbler points out, such an effect would only cause the larger cells to bulge outward, owing to their initial shape.

What becomes of the fluid in the blastocoel during gastrulation is not known. It has been assumed that it is absorbed again by the cells, but no satisfactory evidence for this view has been brought forward.

None of these views, therefore, furnishes a mechanical solution of the problem. Rhumbler, as stated above, pointed to the fact that in a one-layered blastula, such as that of Amphioxus (Fig. 58*a*), the cells are all truncated wedges with the broader base of the cells on the outside. The walls between the cells are approximately radial in direction. If the changes in the shape of cells that take place at the time of invagination be considered, there is found perhaps a clue to the kind of process that is taking place.

When a section through a blastula of Amphioxus just before the time of gastrulation (Fig. 58*a*) is compared with sections of later stages when the yolk-hemisphere begins to flatten and then to turn in (Fig. 58*b*, *c*), a change in shape of the yolk-bearing cells becomes apparent. The inner part of each cell has become broader and the outer part narrower. In other words the cells are now wedge shaped with the broader base of the wedge on the inside. Such a change in shape of the cells would cause the wall to turn in, and the process would continue as long as the bases of the cells continue to broaden.

It can be shown by a mechanical model that the change just

described brings about an inturning of the wall. Rhumbler illustrates this as follows: Elastic steel bands are bent into rings and tied together in a circle as shown in Fig. 59*a*. The rings in one hemisphere are made larger. The rings are mutually compressed. If a solid bar is placed across the inner ends of the larger rings to spread them out, and if at the same time the outer end of each is constricted by a thread, the model invaginates (Fig. 59*b*). The result shows that any process that causes the

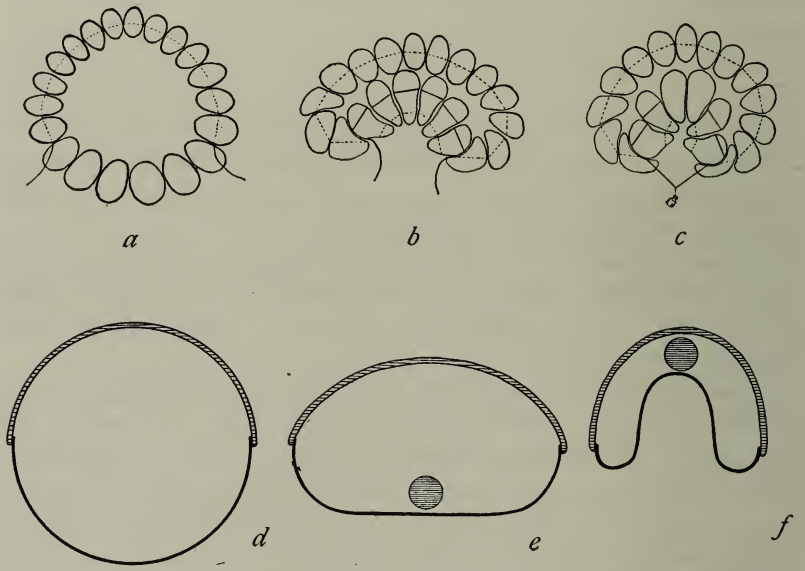


FIG. 59.—*a*, *b*, *c*, model of elastic metal rings tied together to imitate gastrulation processes. (After Rhumbler.) *d*, *e*, *f*, model of paper rings to imitate gastrulation. (After Bütschli.)

cells of one part of the blastula wall to become broader on their inner surface would be expected to bring about gastrulation. There are several agencies that might be invoked to explain such changes in shape of living cells. Surface tension for example. As has been shown in the preceding chapter, the lowering of surface tension at any point of the surface of a drop of oil brings about a movement of the material in the interior toward the region affected, and a superficial out-flow at the surface. Such a change might cause cells that are held together in a plate to broaden out on the affected side. Is it possible then to appeal to such a mechanical explanation as the possible cause of invagination?

What could bring about a change in the interior of a blastula that would lower the surface tension of the inner wall? The two most probable suggestions that may be offered are that during cleavage, substances may be secreted by the cells into the blastocoel that cause the inner ends of the endoderm cells to enlarge when a certain critical stage is reached. Another possibility is that the accumulation of carbon dioxide in the blastula cavity changes the surface tension of the inner ends of the endoderm cells. It is also conceivable that changes may take place in the endoderm cells themselves that make them more responsive to conditions within the blastula cavity.

It may be asked why do the large yolk-bearing cells—the future endoderm cells—turn in rather than the smaller cells of the opposite hemisphere. Rhumbler gives two answers. First, the smaller cells, other things being equal, have a higher surface tension and are less easily changed. Secondly, the consistency of the smaller and larger calls is undoubtedly different. The smaller cells of the polar hemisphere derived from the more superficial part of the egg are also probably more solid than the larger cells of the lower hemisphere.

An attempt to account for gastrulation was made by Bütschli in 1907; it was more fully developed in 1915. Bütschli pointed out that expansion of the inner endoderm wall of the blastula would cause the wall to turn in, but the expansion is assumed to arise from a different set of conditions from those to which Rhumbler appealed. Bütschli constructed a simple model to illustrate the inturning. A thin strip of gelatin (0.048 cm. thick, 2–3 cm. broad and 9.5 cm. long) is bent into a half-circle. A strip of stiff paper of the same breadth and length is bent also into a semicircle. The two pieces are stuck together in a ring (Fig. 59*d*). If now a piece of filter paper saturated with hot water is thrust into the ring near the gelatin hemisphere, this hemisphere flattens and turns in (Fig. 59*e*). The resulting figure is something like a gastrula. The inturning is brought about by the rapid absorption of water by the inner wall of the gelatin strip. Its inner surface becomes greater than its outer surface. This causes the plate to bend as shown in the model. Bütschli did not mean to suggest, however, that the absorption of water by the inner wall of the blastula is the cause of its swelling, but rather that a process of growth of the inner lamellae of the endo-

derm wall takes place which causes an increase in its area over that of the outer surface. He suggests also that in addition to this process, the expansion of the ectoderm half of the blastula wall may also take place at the same time and assist in the process. Bütschli contrasts his own view with that of His ('75). The latter assumed that the rolling in of embryonic plates is due to differences in growth in different parts of the surface. Bütschli points out that such a process will only cause an uplifting of the surface rather than an invagination. The latter will occur only

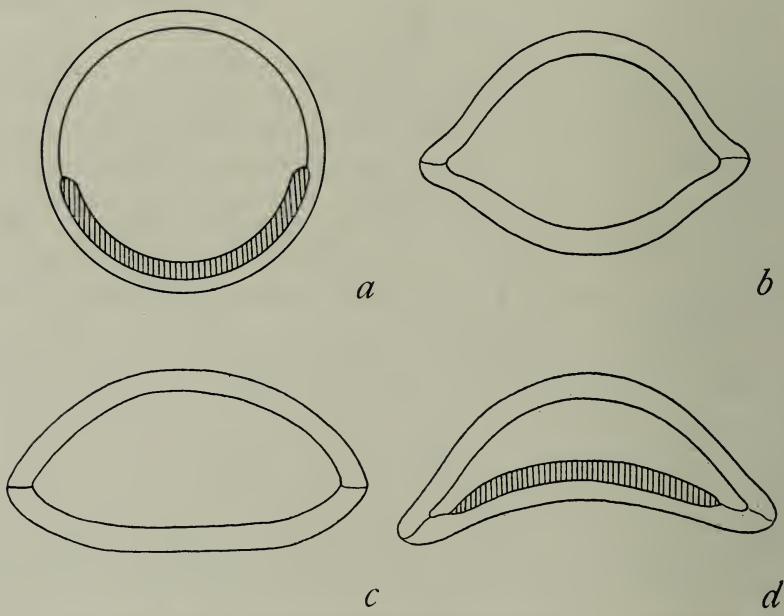


Fig. 60.—Model of gelatin-cups to imitate gastrulation. (After Spek.)

when the growth on the inner surface of a membrane is greater than that on the outer surface.

Recently Spek ('18) has developed further Bütschli's idea and has appealed to the known power of colloids to absorb water as furnishing the factor for inturning. Spek's model consists of hemispheres of agar-agar and of gelatin (Fig. 60*a*). The model is made in the following way: A mixture of 20 per cent gelatin plus 3 per cent agar in the ratio of 3:1 is poured, while still warm, into a space between two cups where it is left until it hardens to form a hemispherical shell whose wall is about 4 mm. in thick-

ness. It is then removed and another similar shell is made. Then on the inside of one of these shells a solution of 20 per cent gelatin is poured, and the shell rotated as the gelatin cools so that the gelatin forms a thin inner layer. The two hemispherical cups are then stuck together to form a hollow sphere (Fig. 60*a*). Water is then squirted into the interior until it is full, and the model suspended in a jar of water. The inner layer of pure gelatin absorbs water faster than does the outer wall of gelatin plus agar; this causes that part of the wall with the inner layer of gelatin to turn into the interior (Fig. 60*b*, *c*, *d*).

Similar models may be made to imitate the inrolling of the neural tube. A cylinder of agar gelatin is made and then along

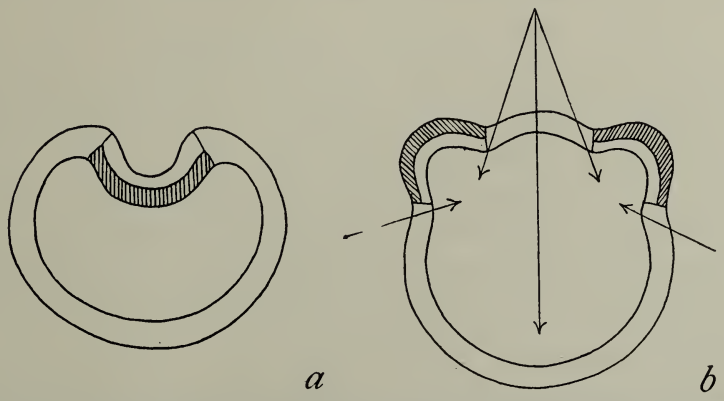


FIG. 61.—*a*, model to illustrate inturning of central nervous system of vetebrates; *b*, model to illustrate pouches from gut of Amphioxus. (After Spek.)

one side an inner layer of gelatin is added (Fig. 61*a*). Placed in water the wall of the cylinder turns in along the region lined by gelatin.

By reversing the position of the layer of gelatin, i.e., by putting two bands of gelatin on the outside of the cylinder (Fig. 61*b*), along two zones, the wall turns out along the two bands, i.e., it evaginates, thus imitating to some extent the two outgrowths that form the coelomic pouches of Amphioxus, etc.

The special merit of this model lies in the fact that protoplasm is known to be a colloidal substance or at least to contain colloids. Colloids have remarkable properties of swelling. Whether regional differences in the colloidal material are traceable to the egg itself, or whether such differences appear in the different parts of the

blastula as development proceeds due to chemical changes taking place is not known. Nevertheless the simplicity of the postulated means to produce in-turning and out-turning of the embryonic layers makes these suggestions well worth careful consideration.

Spek also points out that since the swelling of colloids is greatly affected by soluble substances in solution, the action of salts may be an important factor in development. Colloids swell more in weak acid and alkali than in pure water. Neutral salts also have a strong effect on the swelling and this varies with different salts. Of the alkali salts, those of lithium act most strongly, then comes potassium, sodium, and calcium. Magnesium salts also in a neutral medium cause certain colloids to swell. Spek points out that Herbst's lithium larvae may be explained as due to the surface action of lithium on the endodermal half of the blastula-wall causing the outer surfaces of the cells to swell more than the inner surfaces. As a consequence the gastrula turns out instead of in. This view postulates that the endodermal region is more affected by lithium salts than the ectodermal.

Spek further suggests that the localization action is due in the first place to the entering salt causing a precipitation of the albuminous and lipoid substances of this region. This superficial precipitation prevents or hinders further passage of the salt, but leads here to a localized swelling, etc.

These attempts to apply the properties of colloids and salts to some of the known effects of salts on embryos are ingenious and may give a clue to the effect of salt solutions on the embryo that has been long sought to explain the many kinds of abnormalities that have been observed in these solutions, but as yet such views have little more value than possible suggestions for further study.

The preceding discussion is based on the fact that a change in shape of the inturning cells takes place. The assumption does not necessarily involve any increase in the volume of the cells. A change in surface tension may, however, involve a change in permeability, and water may be either absorbed or given off. If absorbed, the change in shape might be attributed to this factor causing a widening of the cells on their inner surfaces; but the question would at once arise whether the absorbed water would not immediately become distributed equally throughout the cell. If so, its presence could not be used to explain the local

change that takes place during inturning. On general grounds it might be supposed that water absorbed would be rapidly distributed in the cell, and this, if true, would be a serious difficulty for the theory. Whether after a change in surface tension followed by the absorption of water the latter might be locally fixed by one part of the cytoplasm rather than by another, remains to be demonstrated. In the gelatin models the absorption of the water is sufficiently rapid and its transportation through the gelatin so slow that there is time for an inturning before saturation occurs. In living cells on the contrary, the distribution of the water may be too rapid to bring about the inturning observed during gastrulation.

Other Types of Gastrulation

The invagination to form the archenteron takes place in a great variety of ways in different forms. In the ascidians, the yolk-cells are so much larger than the ectoderm cells that they occupy during invagination much of the blastocoel space (Fig. 58*d*, *e*), or rather there is left after segmentation only a narrow arched blastocoel. The change in shape of the yolk-cells—endoderm—is apparent and suffices to explain the inturning.

In other eggs (Bonellia), the ectoderm may lie almost directly on the yolk-cells. The former seem to grow over the latter (epibole) and enclose them in this way. It is quite possible that some such change does take place, but it may be assisted by the change in shape of the yolk-cells that goes on simultaneously. The yolk-cells would then disappear from the lower hemisphere not so much by overgrowth as by a change in shape—the outer ends of the cells becoming narrower as the ectoderm advances. The explanation would be in principle the same as in Amphioxus.

In the frog the gastrulation process is more involved, since there is a large mass of yolk-cells, several layers in depth, to be carried into the egg. The process begins at the edge of the gray crescent where, along a line (Fig. 62*D*), the superficial cells change shape—each cell swelling up at its inner end and becoming pointed at its outer end until these cells are withdrawn from the surface of the egg. The outer layer of cells in front of the dorsal lip rolls around the edge into the interior as the lip of the blastopore advances. A similar change in shape of the cells takes place

in a semicircle (lateral lips of blastopore) below the equator. Simultaneously the yolk-mass sinks in, in front of the advancing lips of the blastopore-rim, until finally all yolk-cells disappear into the interior.

Numerous attempts have been made to study experimentally the movements of cells during the process of gastrulation in the

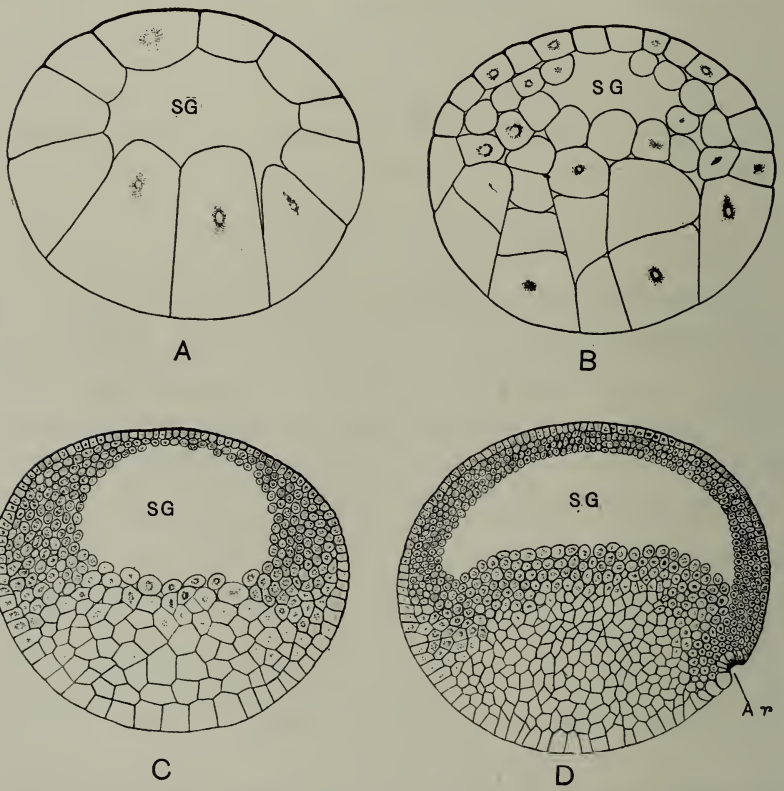


FIG. 62.—Sections of late cleavage stages, and beginning of gastrulation of frog's egg.

frog and other amphibians. The earlier attempts to study the movements by means of injuries (pricking the egg) in various parts of the gastrula are open to objection on the ground that adjustments may take place that obscure the normal series of events. These questions will be considered at another time. A more successful method consists in marking different regions of the egg with dyes of various kinds. This method can be utilized to best advantage on eggs that are not pigmented. The eggs

of some of the urodeles are suitable for such experiments. Experiments of this kind will be considered later.

In teleostean fish and selachians a flange of cells turns under to form the roof of the archenteron. The mechanism of the process has not been worked out. In reptiles a pit sinks down in the surface of the blastoderm and becomes a sac extending forward. Here, we may suppose that the superficial cells over a circular region respond to changes in conditions beneath and invaginate in practically the same way as in Amphioxus.

There are other ways besides invagination by which cells pass from the surface to the interior. The inwandering of mesenchyme cells is typical of such changes (Fig. 21*k*). Individual cells pull away from the surface and, like amoebae, crawl into the interior. The same changes that are involved in the movements of amoeba may be appealed to. It has been suggested that the most important factor in amoeboid movement is a change in surface tension at one side that leads to an axial flow of material to that side where a pseudopodium is thrust forward. From this point of view, inwandering of cells is essentially the same as invagination—in one case a single cell responds, in the other all of the cells of a region respond at the same time.

The question may arise whether changes may not take place within the cell itself of such a sort that the surface tension of the cell is lowered so that it responds before its fellows. Such a process may, indeed, be supposed to take place, and might lead to migration of the cell away from the others. At present, however, we have no explanation as to how such a change could be brought about, although from what has been said in connection with changes from gel to sol in cells during division we are predisposed to think that such changes might take place.

Invagination as a Means of Organ Formation

Foldings of cell-surfaces or of membranes is a very general method by which organ-formation takes place in embryos.[1] For

[1] Gudernatsch ('13) has called attention to the fact that in all processes of folding, in which an epithelial layer takes part, the *basal* ends of the cells form the apex of the fold. The ectoderm folds turn in, and endoderm, when it folds, turns out. In both cases it is the same surface that forms the apex of the fold. These are simple facts of observation and offer no clue as to the mechanism involved. Gudernatsch also points out that when a folding of

example, from the archenteron of Amphioxus two series of gut-pouches are given off dorso-laterally. Similar coelomic pouching occurs when the body cavity and hydro-vascular system are formed in the sea-urchin and starfish. The formation of the optic vesicle, the sinking in of the ear vesicle and nasal pits in vertebrates are all examples of this kind of process. If change in surface tension on one side suffices to explain gastrulation, it will probably explain most or all of these other cases.

In other situations where infolding takes place as in the formation of the central nervous system of the vertebrates the process may be the same, but since the inturning is preceded by a drawing together of surface materials extending at first far out over the blastoderm it is possible that the case may not be quite so simple as in typical gastrulation. But even here a careful examination of preparations or even of published figures shows that, from the first appearance of the broad neural plate with its shallow and broad axial groove, the individual cells of the region affected are changing shape. The inner ends of some of the cells draw away from the surface without losing contact laterally with their fellow cells, so that the ectoderm, at first two layered, may be said to become three, four or more layers deep, even if all the cells still retain contact with the outer surface by means of slender processes. The result is that a relatively wide surface contributes to the material that is concentrating along the axial line, and sinks in as a consequence of the changes in shape or movements of its constituent cells. For, since most of the cells have become broader at their inner than at their outer ends, the whole plate must bend inwards to make room for the increase in the surface of its inner layer. Thus the process is essentially the same as that of gastrulation and the same mechanical principles may be appealed to.

an epithelial layer is in the opposite direction, as when the limb bud pushes out, the ectoderm is carried by a rapid growth of the under-lying tissue, i.e., these are pressure folds. To these cases should be added the amniotic folds of vertebrates and insects which seem to be due, in the main, to the sinking of the embryo into the interior of the "egg." To what extent and in what way the ectoderm contributes to the process is unknown. The allantoic sac of birds and mammals seems to "grow" as a result of the distension of its walls by the pressure of the allantoic fluid inside. It may appear here that the result is due to some peculiarity of the wall that secretes an internal fluid thereby creating a pressure, or to the secretion of some substance by the inner wall that absorbs water, or to the growth of the outer covering layer of mesoderm. Possibly more than one of these factors may be contributary.

Glaser ('16) has studied the changes that take place during the inrolling of the nervous system of the hell-bender (Cryptobranchus allegheniencis) and made some determinations with respect to the outer and inner layers of the inturning neural plate. He has also estimated the relative amount of water in the plate and in the rest of the frog-embryo. There was found to be no increase in the number of nuclei during the period of inturning.

Even admitting an occasional division, which might be compensated for by a slight elongation of the embryo, it is still evident that cell multiplication plays no rôle in the results.[2]

When the number of nuclei in the outer half of the early neural plate, i.e., in the more superficial layers, is compared with the number in the inner half in different stages of infolding it is found that there are relatively more in the outer half in the younger stages, and fewer in the later stages. The averages of the differences are shown in Table XXI for three stages. The result may be interpreted to mean that as changes in the shape of the cells take place, the inner ends become broader than the

TABLE XXI

Distribution of Nuclei in Upper and Lower and Inner and Outer Zones

Stage I, Flat		Stage II, Half-folded		Stage III, Folded	
Upper	Lower	Upper	Lower	Inner	Outer
32	31	31	25	15	40
32	21	22	42	15	45
34	24	16	34	21	52
55	14	27	29	13	34
39	33	18	32	22	47
38	20	27	55	16	43
31	28	33	37	26	38
33	25	29	45	13	38
37	21	21	37	20	32
44	24	19	32	20	35
Ave. 38	24	24	37	18	39

[2] As Glaser points out (Science '16), in other forms such as the chick the published figures indicate an increase in the number of nuclei. Unless the sections were of the same thickness and other conditions taken into account these figures do not necessarily give accurate data. But even if an increase does take place there is evidence showing that inturning may occur without it.

outer ends causing the nuclei to shift inwards. The shift might also be explained if some of the cells draw inwards, thereby increasing the number of cells in the inner half as compared with the outer half of the plate. The results in either case would be the same, and cause the plate to sink inwards.

It was not possible to determine by direct means whether during these changes more water is absorbed by the inner than by the outer superficial half of the plate. Glaser attempted to get some information on this point by weighing the excised nervous system of the frog and of amblystoma at an early stage and comparing the fresh and dry weights with those of the rest of the embryo. The results show that the amount of water in the nervous system taken as a whole during the stage examined is greater than the amount of water in the rest of the embryo, but as he points out, this does not necessarily mean that the water absorbed is present in greater excess in the inner half than in the outer half of the neural plate. The result, therefore, does not furnish evidence that can be used to account for the postulated changes in volume of the inner and outer halves of the neural plate during inturning. Glaser considers Rhumbler's theory of surface tension and does not find it acceptable without certain reservations. He suggests that if liquefaction occurs in the neural plate, this, rather than altering its surface tension, may be an important factor in its inturning. Instead of a lowering of the surface tension, he prefers to assume simply a surface effect. "This does not exclude the factor emphasized by Rhumbler, but leaves room for such other possibilities as liquefaction, etching, and changes in permeability," etc.[3]

Local thickening is perhaps the next most common method of organ-formation. It is often spoken of as though it were due to a local multiplication of cells followed by their enlargement. The formation of the lens of the eye in the vertebrates is a case in point. In some forms and under certain conditions it may begin as an invagination, in others as a solid thickening. The

[3] Glaser also made measurements showing that the nuclei are larger in regions having a higher water content. He uses this evidence in connection with the difference in sizes of the nuclei at the edges of the neural plate and at the center. It is rather uncertain, I think, whether the greater inrolling of the edges may not be due to the sinking in and bending of the whole plate rather than to a more rapid local difference.

fact that it sinks inwards in all cases suggests that a similar kind of action takes place both in invagination and in thickening.

After the initial step by which the beginning of an organ (its foundation or "anlage") is established there follows a period of rapid multiplication of its cells. This growth period calls for no special mechanical principles other than the mutual adjustments of the cells to each other, although, of course, the general activity of cell-division, the keeping apart of the different organs, as well as certain combinations of cell-layers or masses, that bring about interrelations of blood vessels, lymph channels, nerve fibres, etc., within the growing organ, all call for exhaustive study. Relatively little experimental work has yet been done in this field. Some of the evidence bearing on these topics will be considered later in connection with the experimental work on transplantation. For the present we may confine our attention to the possible mechanical processes involved in the initial stages of the organs—stages that for the most part can be traced back to the end of the cleavage stages.

The formation of organs by the lifting up of the edges of a flat plate to form a tube—a process that is conspicuous in the neural tube of the chick and mammal—has the appearance at first sight of being the reverse of the process of gastrulation, especially if the plate is thought of as lifting up and rolling in over a more stationary central axial portion. In reality it is the sinking in of the axial material that is the primary agent in the change, and the coming together of the sides to meet above the groove may be a secondary consequence of invagination. There are no grounds for regarding this change as involving any different process from that seen in gastrulation. Similarly for the folding of the endodermal plate in the mammalian embryo to make the digestive tube. The axial portion may be regarded as sinking in, while the sides are pulled together as a result. Hardened preparations are often distorted and may give the impression that the axial material is stationary while the sides push across the middle line. This may be misleading.

One of the earliest attempts to give a quasi-mechanical explanation of organ formation was that of Wilhelm His ('74).

In a remarkable book entitled "Unsere Körperform" he approached the study of embryology from a very different point of view from that in vogue at the time. This book is one of the

earliest attempts to escape from the historical treatment of the problem of development—a method that was in full swing in His' time under the guidance of Haeckel and other popular writers, a movement that has continued to the present time but with a gradual lessening of interest and importance.

His tried to show that the foldings of the germ-layers at the time of embryo formation—foldings such as those seen in the development of the neural plate and archenteron of the chick and mammal—can be closely imitated by stretching an elastic sheet (of rubber for example) at fixed points. He suggested, therefore, that localized growth in certain regions or at certain points of the elastic blastoderm might be responsible for the folding of its surface. It need not be denied that growth or increase in mass at one region would cause an elastic surface to be wrinkled or folded, and it is possible that some of the shifting seen in certain cases may be ascribed to such agents. In general, however, it may be said that more complete information concerning the changes taking place when a layer is folded do not support His' view, for, it turns out that what takes place is not so much due to local thickening as the drawing together from over an extensive region of material (cells) that involves a sinking down of the material in certain regions and not to its elevation above the general surface. In other words, the changes that take place seem more in accord with the principles appealed to by Rhumbler than those invoked by His. Local growth may, it is true, take part in this formation of organs, but this follows rather than precedes the changes that lead to the initial separation of an organ from the rest of the embryo.

While we have not advanced very far in an understanding of the so-called formative influences that determine the changes in shape in different parts of the embryo, nevertheless there seems to be nothing in the changes that is beyond the range of explanation of ordinary physical and chemical processes. If by means of surface tension and of swelling we can account for so many of the initial steps in organ formation it seems not unreasonable to expect that a fuller knowledge of the other changes may furnish equally simple solutions. It is a lack of knowledge of what occurs that is more apparent than that these changes are beyond the reach of a physico-chemical explanation.

Shifting of the Materials During Gastrulation in Amphibia

It has for a long time been realized that extensive movements of the cells take place during gastrulation. This was obvious in forms in which one hemisphere is inturned and also in forms whose cell-lineage had been studied in detail. Even in amphibians, reptiles, and birds in which a large number of cells are present before gastrulation, there was a good deal of information concerning the changes taking place during gastrulation. But in the amphibia, in particular, much divergence of opinion existed as to the extent of the changes that take place at this time. The older method of inference based on sections of preserved material proved inadequate to give the required information.[4] A new procedure was inaugurated when, by means of staining localized regions of the blastula and gastrula, the subsequent movements of the surface materials could be followed. The egg of the frog is unsuited for experiments of this kind owing to the black pigment covering the upper hemisphere, but the eggs of some of the urodeles, that have little or no pigment, are more suitable for this sort of work.

The first extensive marking experiments were made by Goodale ('11), who followed the movements of the different regions of the egg during gastrulation by marking the blastula and early gastrula stages of the egg of Spelerpes bilineatus with Nile-blue sulphate. Preliminary observations had shown that this dye does not injure the living cells and that spots of it are retained for several days. The outer jelly was removed, the inner membrane pricked to allow the escape of the perivitelline fluid, and a small drop of dye applied to the outer membrane at any desired part of the egg. The dye penetrates the membrane and stains a spot beneath. When this has been accomplished, the egg is returned to water and the excess of the dye washed off.

An egg, marked by six spots above the equator, a few hours before the dorsal lip appears, is shown in Fig. 63*a-d*. The spots

[4] The main facts were fairly well established (Jordan '93, Morgan '94, '97, '03, Eycleshymer '98, Kopsch '95, King '02). Several earlier observers made use of pigmented spots on the normal egg as indices of cell movements (Kopsch '95, H. V. Wilson '00). Several attempts were also made to trace the changes taking place by causing local injury to the egg (Ikeda '02; Moszkowski '02; King '02; Morgan '02; Todd '04; Assheton '95; Brachet '03).

hold the same position until the dorsal lip appears. Then as it develops, "a remarkable series of changes occur." The spots become elongated bands extending to and into the lips of the blastopore (Fig. 63). Not only is this apparent over the region of the future neural folds, but laterally and especially ventrally as well. So extensive are the latter movements that all of the yolk hemisphere must be supposed to pass into the interior. These and numerous other experiments led Goodale to conclude that in Spelerpes the dorsal lip itself moves ventrally much less than does

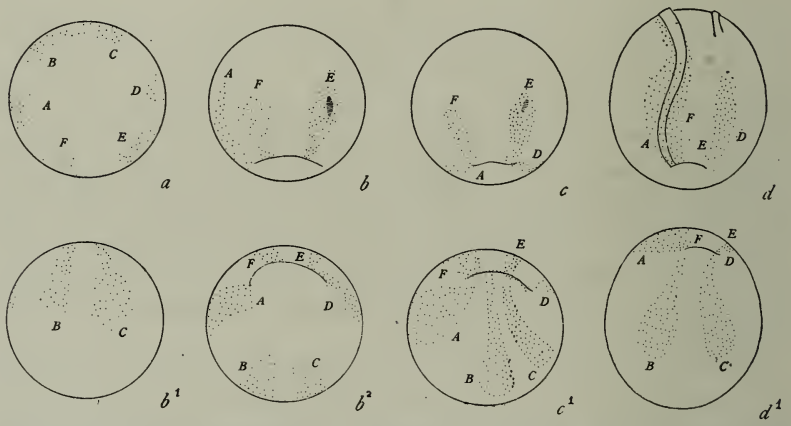


FIG. 63.—Gastrulation of Spelerpes. The dotted regions (*A*, *B*, *C*, *D*, *E*) in these figures indicate stained regions and show the movements of the regions during the closure of the blastopore. In *a*, the egg was stained in six places above the equator; the egg was about to begin to gastrulate. In *b*, *c*, *d*, the movements in front of the dorsal lip are shown, as indicated by the dotted regions, *A*, *F*, *E*, *D*. In b^1, b^2, c^1, d^1 the movements over the yolk-field are shown, as indicated by *A*, *B*, *C*, *D*. (After Goodale.)

that of the frog. After the initial elongation, the marks above the dorsal lip undergo a further extension when the embryo begins to lengthen.

Bertram Smith ('14) by staining with Nile-blue, made many observations on the movements of cell regions during gastrulation of the large white egg of Cryptobranchus. His results confirm those of Goodale in all respects. More recently Vogt ('22, '25, '26) has carried out similar experiments with the eggs of Triton by localized staining with Nile-blue, and Goerttler ('25) working under Vogt's direction has confirmed and extended the same experiments.

The eggs were stained by the application at the surface outside the membrane of small pieces of agar saturated with the dyes. An egg was stained by alternate red and blue spots in a half-ring, as shown in Fig. 64, at the time when the dorsal lip appears. The stained areas later became part of the medullary folds (Fig. 64*c*, *d*). The spots were drawn out into bands both before and after they were incorporated in the neural folds. Another egg was

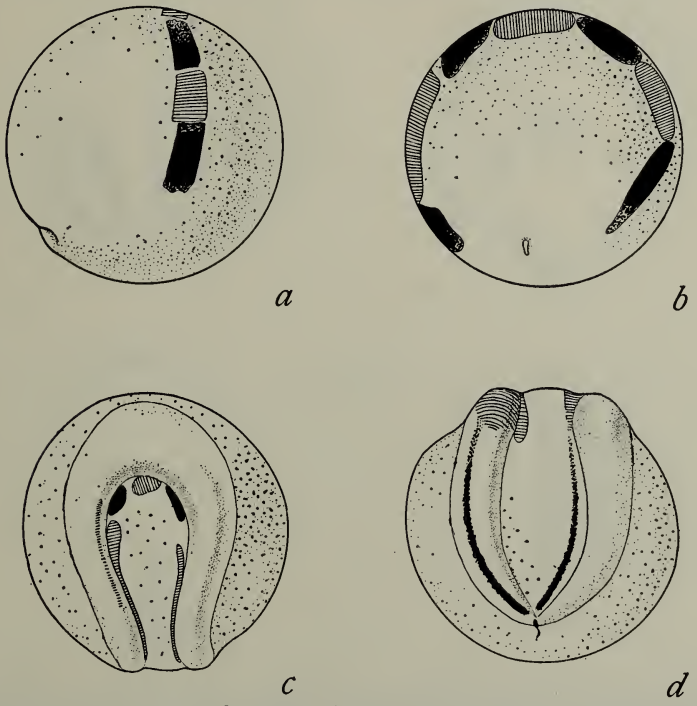


Fig. 64.—*a*, *b*, young gastrula stages of Triton that have been marked by stains of two colors in a half ring extending nearly half way round the egg; *c*, *d*, the subsequent position of these marked areas. (After Goerttler.)

stained as shown in Fig. 65*a*. As gastrulation went on, the spots anterior to the dorsal lip became much elongated toward the dorsal lip (Fig. 65*b*), while those at the sides were brought more nearly to the middle line, and at the same time were greatly elongated. The presumption from this evidence is that some of the material anterior to the dorsal lip passes into and under the rim. While this is taking place the more anteriorly lying surface material is correspondingly drawn out into streamer-like bands.

The diagram (Fig. 66*C*, *D*, *E*) shows what is supposed to be taking place. The material just in front of the dorsal lip is turned in to become the notochord, while that to the right and left becomes mesoderm.

Vogt's interpretation of the gastrulation process is not different from that held by several students of amphibian development. Surface material (endoderm) is first turned under at the

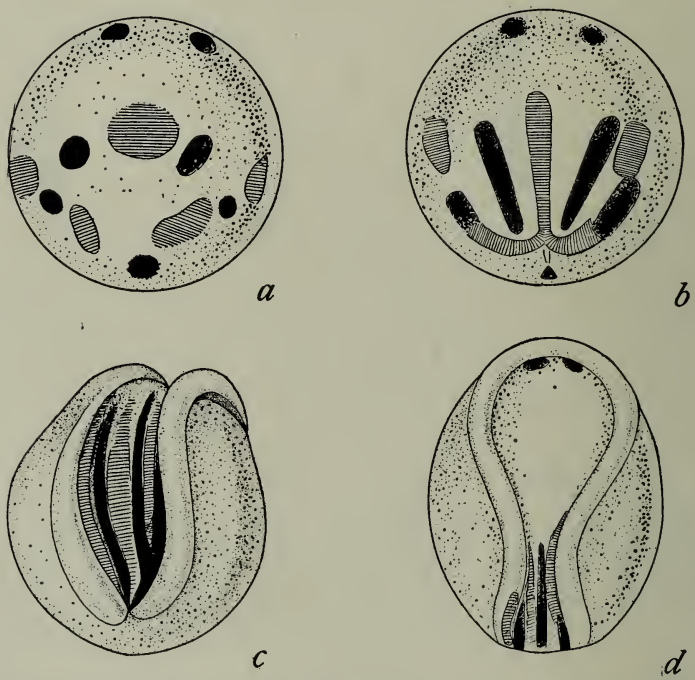


Fig. 65.—*a*, young gastrula of Triton marked with two stains in the upper hemisphere; *b*, the changes that take place during gastrulation are shown by the shifting of the stained regions; *c* and *d*, the position of the marked areas when the neural folds have appeared. (After Goerttler.)

dorsal and lateral rim of the blastopore to become the notochord and mesoderm (Fig. 66*A*, *B*). While this is taking place, the yolk hemisphere is also moving forward beneath the rim to form the floor and sides of the archenteron. Later the sides grow up beneath the chorda-mesoderm sheet to form the new roof of the archenteron. The chorda-mesoderm layer comes to lie between the new roof and the outer layer of the embryo. Vogt's view of the position of the superficial layer of the embryo at the

moment of gastrulation in terms of their final position in the embryo is illustrated in Fig. 66*A*, side view, and Fig. 66*B* viewed from below. It will be seen from these diagrams that somewhat more than half of the upper hemisphere is covered by dorsal ectoderm that will form the neural plate. The remainder of

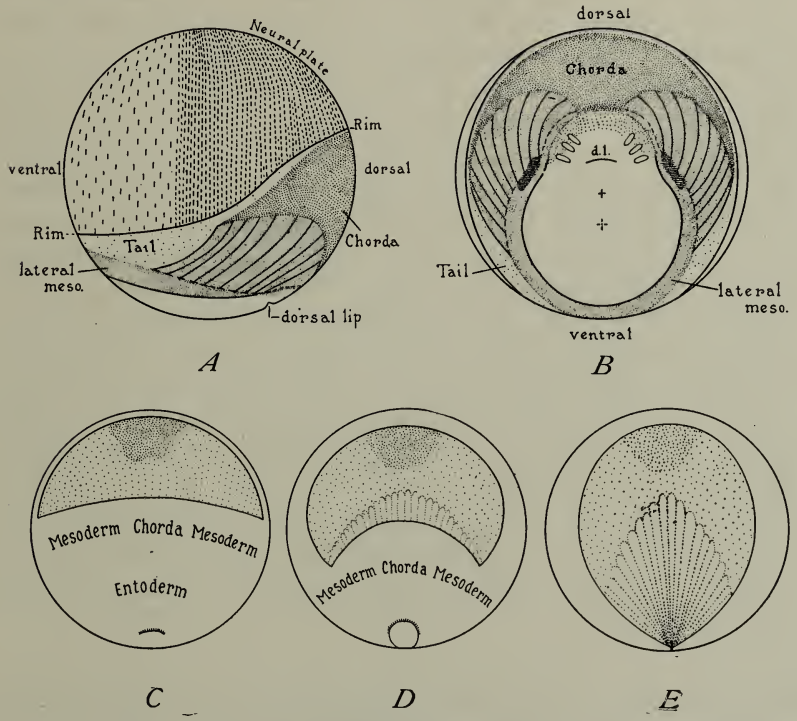


FIG. 66.—*A* and *B*, diagrams of the areas at the surface of an embryo of Triton at the time when the dorsal lip of the gastrula has just appeared; *A*, in side view; *B*, as seen from the yolk pole. (After Vogt.) The three lower figures, *C*, *D*, *E*, show the region of the prospective neural plate (stippled), and of the chorda and mesoderm in front of the dorsal lip. These figures also indicate the elongation of the materials of the neural plate during the closure of the blastopore when the chorda and mesoderm are turned into the blastopore lip. (After Goerttler.)

the ectoderm of this hemisphere (less than half of it) becomes the surface layer of the embryo. A curved line ("Rim") in the diagram indicates approximately the limits of this area. The material for the notochord occupies the area immediately above the dorsal lip and spreads out halfway around the egg, as a broad crescent.

Between this material at the sides and the prospective rim of the blastopore lies on each side the material for the mesoblastic somites of the trunk region (parallel curved lines in the diagram). This is the material to be turned under at the lateral rims of the blastopore. Coextensive with these lateral fields, and immediately at the lateral and posterior rim, is the material for the lateral mesoderm. It is the first material to be turned under at the rim. Finally the material for the tail region (ecto- and mesoderm) lies just below the ectoderm (lightly stippled in Fig. 66*A*, *B*). The regions of the future gill-slit material, and of the forelegs are indicated in Fig. 66*B*. It need not be supposed that all of these areas are as predetermined as these diagrams indicate. It is more probable that there is considerable latitude possessed by the regions whose exact fate is in part determined by the final position they reach in later stages. The diagrams only serve to indicate in a general way what the different materials, lying on the surface, will become, in the course of time, when they develop into the special organs of the embryo. The picture, here given of the course of events, is more definite and precise than that suggested by previous evidence from normal development and from that obtained by more artificial methods of various kinds. With the exception of about a quarter of the surface material, that forms the surface layer of ectoderm, all the rest is turned into the interior to form the central nervous system, mesodermal organs, and archenteron.

Mutual Influence of the Inner and Outer Layers on Each Other

In the preceding account of the gastrulation process, the possible influence of the coöperation of the parts has been largely ignored. If the materials on which the changes rest are thought of as graded radially around a center or as held in check from the periphery inwards, the unity of the reaction may appear to be insured, but it may seem questionable whether this takes into account all the factors involved in such a process even as simple as gastrulation appears to be. In fact, experimental embryology has revealed other cases where the influence of one part brings about an invagination of another part, the inturning resembling in its physical aspects such a process as that of gastrulation or

of other forms of pouching. The evidence comes from three main sources: (1) the development of the lens of the eye, (2) the inturning of the external gill-slits, (3) the inrolling of the central nervous system. The experimental work that relates to these topics will be more fully treated in another place, but the evidence may be considered here, in so far as it bears on the problems under discussion.

The lens of the eye of the frog develops as a thickening of the under layer of cells of the ectoderm exactly opposite the end of the optic tube or vesicle that pushes out from the inter-brain. The withdrawal of a group of cells from the inner layer of the ectoderm that sinks beneath the surface to form a sphere of cells, is essentially the same kind of process, if we may judge from appearances, as that which occurs in other invaginations. Sometimes, in fact, the cells sink in as a cup to round up afterwards into a solid sphere by the inturning of the margin of the cup. Lewis in 1904 first showed that the location of the lens is determined by the optic vesicle, and is not an independent function. This was done in one way by cutting off the end of the vesicle and inserting it beneath the ectoderm of some nearby region of the head of the same or of another embryo. The ectoderm lying above the inserted vesicle thickened and formed a lens. In another way Lewis also obtained similar evidence. The ectoderm above the optic vesicle of an embryo at a stage just before the lens is to form, was removed and a piece of ectoderm from another part of the body, or from another embryo, or even from an embryo of a different species (whose skin had a different color, hence could be identified more completely) was grafted in place of the excised piece. The new ectoderm became immediately incorporated, and from its inner surface a lens thickening developed. These results have been confirmed by several later experimenters in all essential respects. There is general agreement that the presence of the optic cup calls forth the development of the lens in the overlying ectoderm. The experiment does not reveal the nature of the influence, whether it is a contact reaction, or an action at a distance, possibly through the diffusion of some chemical substance emanating from the vesicle, or conversely by its withdrawal or counteraction of certain materials in its vicinity. However the effect is produced, it is evident that the location of the lens is in the main determined by the presence of the optic vesicle. If that influence

is chemical in nature, as seems to be most probable, the nature of the response may not differ in kind from the influence of the interior of the blastula that causes its wall to invaginate. The changes that take place in the lens-cells, subsequent to its induction by the optic vesicle, introduces other questions that need not be considered here.

In later stages of development of the frog embryo, there appear on each side of the neck three vertical slits due to inturning of the ectoderm. Ekman has shown that when the ectoderm of the gill-slit region is removed at a very early stage, and other ectoderm from a correspondingly young embryo is grafted over the region, vertical slits will appear in the ectoderm opposite the endoderm slits that have meanwhile developed beneath. There can be no doubt that in these cases also that the inturning of the ectoderm has resulted from influences that come from another region of the embryo, the endodermal folds.

Amongst the early experiments made by Lewis ('07) on amphibian embryos was one in which pieces from the margin of the dorsal lip of the blastopore were cut out (including prospective mesoderm and endoderm) and transferred into pockets beneath the skin of older embryos. Lewis found that these pieces produced a nerve tube, notochord, and muscle somites. The pieces seem to have been grafted too deep in the skin of the recipient embryo to produce changes such as those found later by Spemann and his students that will be described below, or else the origin of the neural tube from the overlying ectoderm was overlooked. The recipient may have been too old, but, if so, it is not obvious why any neural tube should have formed at all, since in the earliest stages of gastrulation there is no ectoderm at the edge of the blastopore lip. Lewis concluded: "It is evident from this experiment that tissue from the dorsal lip of the blastopore possesses great power of self-differentiation, is already predetermined, and does not need the usual normal relations with the rest of the embryo for its differentiation. . . . It is possible that by the transplantation of small pieces or even groups of cells from younger and younger embryos that the localization of the primary organs or tissue-forming substances can be traced back step by step to determine in their early stages correlations necessary for the formation of secondary tissue, or for the differentiation of these."

A series of grafting experiments inaugurated by Spemann ('18, '19, '21) and carried further by Spemann and Hilde Mangold ('24), O. Mangold ('24), Marx ('25), Geinitz ('25), and Bautzmann ('26), have shown that the neural plate of Triton and of the frog may be called forth by underlying tissues consisting of presumptive chorda-mesoderm. The evidence is derived from several kinds of experiments. In its simplest form the experiment is carried out by removing a small piece of the wall just anterior to the dorsal lip of the blastopore and inserting it elsewhere in another younger embryo. The piece is inserted, not in the prospective neural plate region of the host, but far

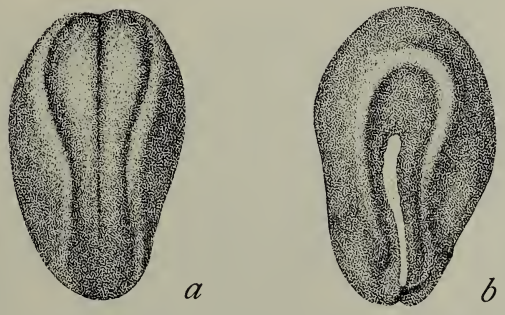


FIG. 67.—*a* and *b* show the two sides of an embryo and its secondary; a piece of prospective endoderm had been implanted into the ventral side of a gastrula. The figure to the left, *a*, shows the normal primary neural plate, and the figure to the right, *b*, shows the secondary neural plate that has arisen in the region of the transplant. (After Spemann.)

to one side, or even in the ventral region. The piece (graft) contained materials that, if left *in situ*, would have become part of the notochord and mesoblastic somites. If the piece were taken to one side of the future median line it might lack the presumptive notochord cells, but contain the mesodermal material of one side.

The graft, after becoming incorporated in the wall of the host, sinks beneath the surface, and later induces in the surface ectoderm of the host a neural plate that extends "forward" from the place of insertion, and perhaps backwards also, due it seems to the extension of the introduced materials (Fig. 67*b*). When the operation is performed in this way, it appears that the entire graft, including its superficial layer of cells, sinks beneath the surface. It may be recalled in this connection that in the normal embryo the superficial layer of cells at and in front of the dorsal lip is

also turns into the interior of the embryo, and does not form any part of the future neural plate, but becomes notochord and mesoderm. In the grafting experiment, the same process seems to occur, and the neural plate, that later appears in front of the invaginated graft, comes from the local ectoderm of the host.

The embryo with two neural folds, one on each side, drawn in Fig. 67*a*, *b*, was produced in the following way. A small piece from the dorsal lip of the blastopore of Triton cristatus (that is lighter in color) at the beginning of gastrulation had been

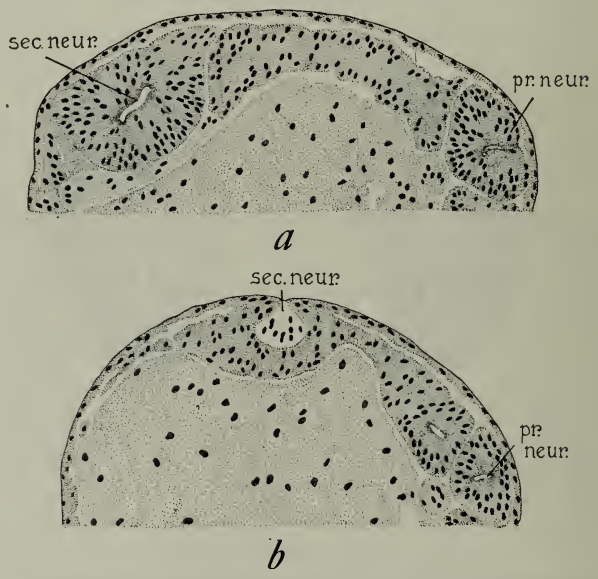


Fig. 68.—Two sections through the embryo shown in Fig. 67. The first section, *a*, is through the anterior end in front of the transplant. The other section, *b*, is further back through the transplant showing the secondary neural folds. (After Spemann and Mangold.)

transplanted into the indifferent ectoderm of an embryo of Triton taeniatus at the same stage of development. Later, as shown in the figures, a normal primary neural fold (Fig. 67*a*), arose on one side, and another secondary medullary fold on the opposite side (Fig. 67*b*). The lighter colored graft, that has become elongated, shows through the overlying neural plate of the secondary embryo. Cross-sections through an older stage of the embryo are shown in Fig. 68*a* and *b*. The first of these is through the anterior end beyond the whitish region of the graft. The

primary neural tube is seen at the right, and the secondary at the left. The other section (Fig. 68*b*) is through the middle region. The closed primary neural tube is seen at the right and the secondary above. The lighter colored piece in the center of the neural tube is the graft (T. cristatus).

The embryo drawn in Fig. 69 was produced by inserting a piece of cristatus from the dorsal lip of an older gastrula into a taeniatus gastrula of the same age. The primary neural tube is seen in Fig. 69*a;* and the secondary in Fig. 69*b*. A later stage is drawn in Fig. 69*c*. The primary embryo shows eye-

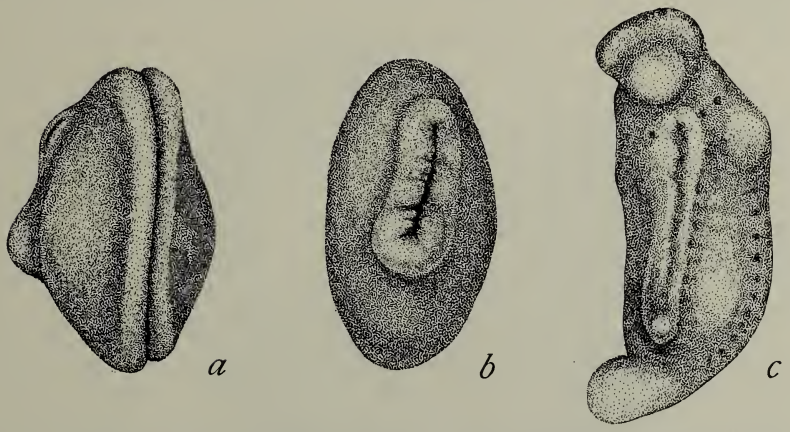


FIG. 69.—Two stages in the development of a double embryo of Triton in which a piece from in front of the dorsal lip of one gastrula had been implanted on the ventral region of another gastrula. The primary embryo is shown in *a*; the second in *b*. An older stage showing both embryos is shown in *c*. (After Spemann and Mangold.)

vesicles, ear-vesicles, and tail-knob. The secondary embryo, lying along one side of the primary, later developed ear-vesicles at the same level as those of the primary. Cross-sections of the embryo (Fig. 70) show the secondary neural tube (to the right) and near it in the section one of its ear-vesicles is present. At a more posterior level, the sections show a distinct notochord beneath the neural tube, and well-developed mesoblastic somites on one side. The pronephros is also present on both sides. At the anterior end the other ear-vesicle is present. The other or primary embryo was normal in all respects.

Further and more detailed experiments have been carried out by Mangold ('24), Marx ('25), and Geinitz ('25), whose work furnishes additional details concerning the potentialities of the

different regions of the gastrula. In some of these experiments a piece of the dorsal lip is inserted, through an incision made in the upper hemisphere, into the blastocoel of an embryo in a somewhat younger stage. It becomes implanted in a part of the inner wall. Later, a neural fold appears from the ectoderm of the roof on the side of the segmentation cavity beneath which lies the inserted fragment which has differentiated into endoderm (?), notochord, and mesoderm. The ectoderm of the host has produced the cells of the secondary neural plate. In another experiment, carried out by Geinitz, the ectoderm is first lifted

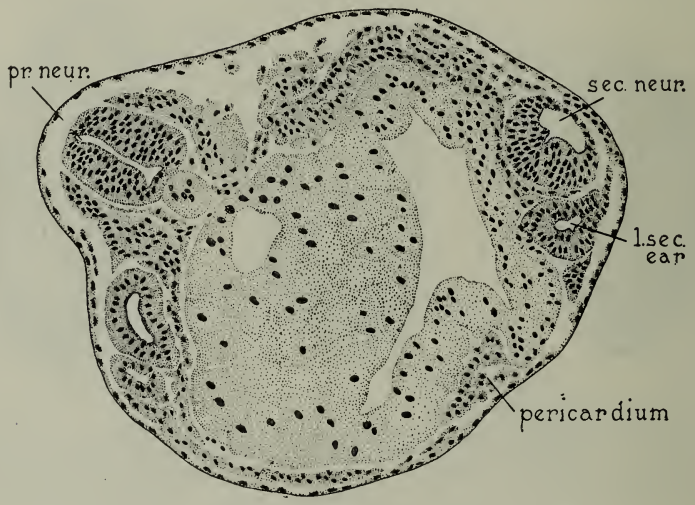


Fig. 70.—Cross-section of embryo drawn in Fig. 69, *c*.

off from a region anterior to the blastopore. The underlying layers are then cut out and inserted inside the blastula cavity. The result is the same as before. A neural plate develops later from the ectoderm of the host (Fig. 180*b*).

The results of these grafting experiments show that any part of the outer layer of cells (ectoderm) of the upper hemisphere can be made to roll in to produce a neural plate by the presence beneath it of materials from the dorsal lip of the blastopore. These materials that normally lie just above the dorsal wall of the archenteron, are generally designated as chorda-mesoderm. Chorda-mesoderm comes from the outer layer cells that are turned under the lip of the blastopore and comes to lie under the neural

plate. The first material turned under at the dorsal lip probably becomes the fore-gut in front of the notochord. The permanent or secondary dorsal wall of the archenteron extends upward from the sides (yolk cells) beneath the mesoderm. Some at least, of the grafting experiments demonstrate that the graft, when prospective chorda-mesoderm, sinks beneath the surface to become the chorda-mesoderm of the secondary embryo. Either the chorda or the mesoderm (or both) is supposed to be responsible for the inrolling of the ectoderm to produce the neural tube which may be presumed to be coextensive with the underlying layers or even to extend beyond them. As yet, however, the details of these relations have not been fully worked out, but as to the main result there can be no doubt.

It appears from these experiments of Spemann and others, that the materials of the egg that lie in the region of the gray crescent constitute the organizing center about which the rest of the egg, that has up to this time remained partly indifferent or undetermined, become molded into an embryo.[5] Spemann has called this dorsal lip region the "organizator," meaning that it acts as a center of influence for the rest of the egg. It might appear, without further explanation, that the organizator has a mysterious influence on the neighboring parts, but the evidence so far at hand would not seem to justify such a conclusion; for, as Spemann has pointed out, the influence seems to be due to the presence of the cells, underlying the neural plate cells, that are destined to produce the chorda-mesoderm. The changes that take place during the sinking down of the dorsal lip material (chorda and its accompanying mesoderm) would appear to be no other than those which take place at the lip of the blastopore of the normal embryo. The specific discovery is that the formation of the neural plate depends on the presence beneath it of chorda-mesoderm, and that the ectoderm of the upper hemisphere is totipotent in its responsiveness to this material.

In relation to the mechanics of invagination the results of these experiments furnish no further information, except in so far as they suggest at least that the response of the ectoderm may depend on chemical substances, perhaps organic substances, rather than on such simple inorganic substances as H_2CO_3 or other

[5] The effect of removal of this region of the blastula by pricking has been described by Vogt ('22).

acids, as has been suggested by Rhumbler and others. It may even be true that the influence is one of contact (stereotropic), i.e., physical, in the case of the neural plate, although such an agent could not be appealed to in the case of gastrulation.

CHAPTER XIV

THE ORIGIN OF ASYMMETRY

It has been found that the median plane of symmetry of the embryo, when not impressed on the egg by its stiff, enveloping membrane, may in certain cases be traced back to another external agent, such as the path of entrance of the spermatozoön, or possibly, in one case, to pressure acting on the egg in the oviduct. It was not found necessary, therefore, to assume that a bilateral structure is present in the protoplasm of the unfertilized egg, as long as its origin could be explained as arising from extraneous sources. There is another group of cases in which structures that are asymmetrical develop. It is as important to discover the origin of asymmetry as to find out how a bilateral symmetry arises in the egg, and the situation is all the more interesting because asymmetry is generally imposed on forms that have a fundamental, structural bilaterality.

The Asymmetry of Flat Fish

While most fish keep the dorsal side uppermost, flounders and soles lie on the bottom of the sea on one side. The anterior end of the body in particular is very asymmetrical. Both eyes lie on the side that is uppermost. The change in position in one eye is paralleled by extensive changes in the shape and arrangement of the bones of the head. Other organs than the head are also much affected; in fact, evidences of asymmetry may be found throughout the entire body. Yet the young flounder is, at first, like other fish. It has a bilateral form and swims with its dorsal surface uppermost. When a certain stage is reached, the young fish sinks to the bottom, but even before doing so the changes that lead to its later asymmetry have begun to appear.

Some species lie on the right side (summer flounder); others on the left (the halibut). Rarely a flounder is caught that is

"reversed." Such cases may seem to indicate that at the beginning both sides have the same potentiality. This view, if true, would still leave unexplained why certain species, with the rarest exceptions, turn onto a definite side—right or left, as the case may be.

The changes that take place when the asymmetry appears have been examined by several embryologists, Pfeffer ('86), Williams ('02), Mayhoff ('14), but the fullest account is that of Kyle ('21) who finds the earliest evidence of asymmetry in the twisting of the gut, and in the position of the swim bladder and other visceral organs. The twist gradually extends forward as the embryo turns to one side, and finally the head becomes involved. Kyle points out that in the earliest stages the asymmetries are not unlike those found in other fishes, but in these a compensation takes place enabling them to retain a dorso-ventral position. In the flat fishes the changes progress until the effects are far-reaching. Kyle seems inclined to think that these changes in the young fish have forced the flat fishes to adopt a bottom life, rather than that the adult stage is an adaptation that has, so to speak, affected the younger stages in the course of evolution. If the changes are germinal to-day, we must suppose that they have always arisen in this way, although not all at the same historic moment unless one adopts the view of the inheritance of acquired characters. In other words, it is not improbable that the present condition of flat fishes has gradually resulted from alterations of the germinal material of such a kind that in the course of time the asymmetry has become more perfect, so that to-day the fish are better adapted to a bottom life. Kyle thinks that the flat fishes are polyphyletic in origin, i.e., this kind of change has occurred independently in at least four groups of marine fishes. Such a view, he states, is supported not only by comparative anatomy, as others had already pointed out, but also by the evidence from embryology, indicating that in each group the changes have taken a somewhat different course.

The most remarkable alterations are those involving the bones of the head, but these changes are always preceded by earlier ones in the head and body. In the skull the changes are "obviously due to pressure or stresses; apart from the tendency to grow, the structures are quite passive. The eye is not pulled into its new position by the frontals or its own muscles; it is demonstrably pushed over by the growth of the subocular ligament or the

prefrontal. These, again, are constrained to grow obliquely by outside pressure. When rupture of the tissues is apparent, it is not due simply to the structures growing apart; they are definitely forced apart." Sinistral forms have almost invariably an air-bladder lying more or less on the right side. Dextral forms have either no air-bladder, or an air-bladder more or less on the left side. "Throughout development, variations in the rate of metamorphosis can be judged by differences in the balancing conditions of the abdominal region."

Aside from the experiment of Cunningham ('91) on the effect of illuminating young flat fish from below that had already turned to one side, there is as yet no experimental work on these fish that bears on the question of their turning. In another group, however, the Amphibia, the asymmetry of the heart has actually been traced to the initial stage, and there is also some experimental work that promises to throw some light on conditions that may influence the direction of the twisting.

The Artificial Production of Situs Inversus Viscerum

Spemann ('06) discovered that if a square piece of the neural plate including the underlying roof of the archenteron of the embryo of the frog is cut out and is then turned round through 180 degrees and reimplanted (Fig. 71*a*), the twisting of the digestive tract (Fig. 71*c*, *d*) and of the heart may be "reversed." Such a result was unexpected, since only a part of the roof of the digestive tract was reversed, and the heart lies on the ventral side of the body. The result might be accounted for if the reversion of the digestive tract is first determined by the reversion of the dorsal piece, and this influence affects secondarily the position of the heart. Spemann suggested, in fact, that the asymmetrical position of the liver, which arises at an early stage from the ventral wall of the digestive tract, might influence the direction of the blood that enters the posterior end of the heart in such a way that the direction of its twisting is affected. If the position of the liver is changed by the operation, the blood current might also be changed, and bring about a reversal of the twisting of the heart tube.

The material collected by Spemann was turned over to one of his students, Pressler ('11), for further study. There were 19

operated embryos, three of Rana esculenta and sixteen of Bombinator igneus, the latter giving a tolerably complete series of stages. In order to determine what condition underlies the normal position of the viscera (situs viscerum) young stages were

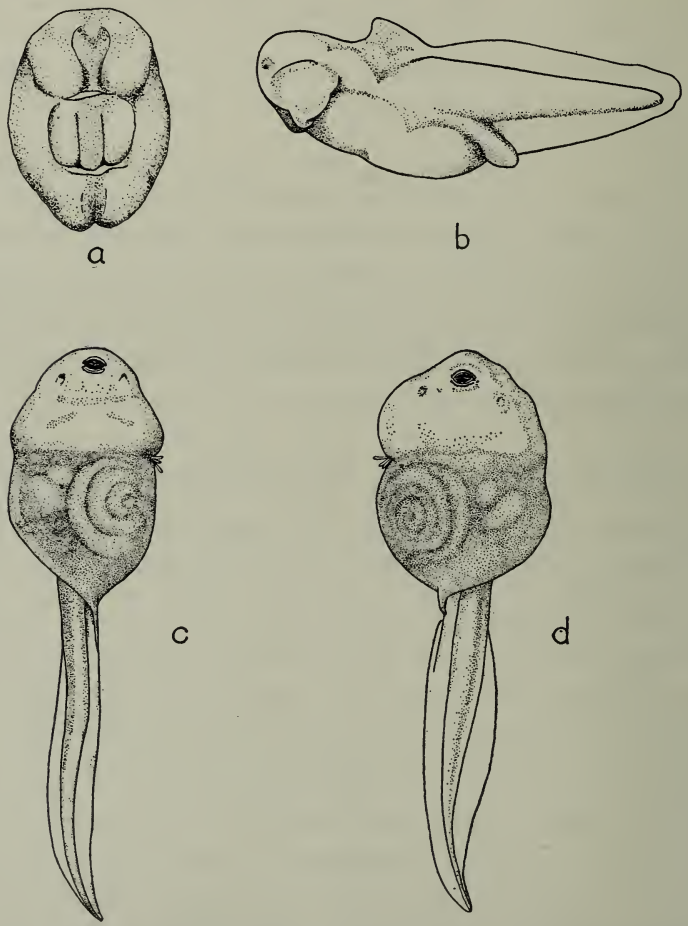


FIG. 71.—*a*, dorsal view of an embryo of a frog, in which a piece of the neural plate has been reversed; *b*, later stage of *a*, as seen from the side. Two older tadpoles, *c*, *d*, one normal, the other showing situs inversus. (After Meyer.)

examined in serial sections. The first appearance of asymmetry is found in a stage where the digestive tract is still a straight tube. On its ventral wall, where its wide pharynx cavity narrows to become the oesophagus, an outpushing marks the beginning

of the liver. Just in front of this, under the lower wall of the pharynx, the beginning of the heart appears (Fig. 72*a*). At this time it consists of a straight endothelial tube lying between walls of mesoderm that have opened out, right and left, to mark the beginning of the pericardium.

The liver rudiment projects a little to the right side of the middle line, and lies just behind the heart (Fig. 72*a*). The intestine turns a little to the left side. The two large yolk-veins that open into the posterior end of the heart are present at an early stage. They are developing, in fact, while the changes outlined above have been going on. The left vein is larger than the right, the smaller size of the latter being due, possibly, to the

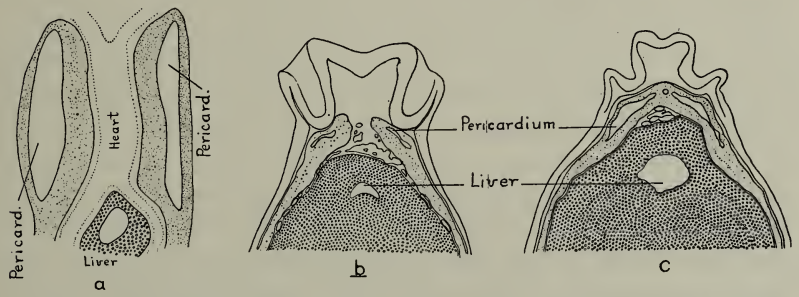


FIG. 72.—*a*, diagram of a horizontal section through the heart region of a frog's embryo when the endothelial tube of the heart is still straight; *b*, horizontal section through the prospective heart region of older stage; *c*, same as last. (After Meyer.)

diminished space on the right side where the liver is pushing out. The pericardial cavities are also at this time a little asymmetrical. The right is slightly smaller than the left. The mesocardium turns above and anteriorly to the right, behind and below to the left. This leads to, or is involved in, the slight bending of the endothelial tube leading through an S-shaped course to the later anticlockwise spiral of the heart tube.

The embryos that develop after the operation (i.e., after the reversal of the dorsal piece) show the following conditions. The dorsal fin does not gradually increase in height from its anterior end to the tail as in the normal tadpole, but is broken in its course (Fig. 71*b*), being higher at the anterior end of the transposed piece and lower at the posterior end, where it joins abruptly the higher fin behind. Evidently the fin has developed as though still in its normal position. Similarly, the medulla and

the chorda of the transposed piece, although continuous with the medulla and chorda of the embryo, change in size abruptly at the anterior and posterior levels of union.

The operation removed the roof of the archenteron. The portion removed includes that part that becomes the dorsal wall of the foregut (duodenum) and the first part of the midgut and may include the region from which the dorsal pancreas is given off. This portion of the gut is then inverted, that is, it is expected to take the opposite turning from that taken by the normal embryo. This initial inversion in the anterior region may be expected to extend backwards throughout the rest of the digestive tract, into regions lying posterior to the inverted piece, provided its course is determined by changes that start in the more anterior region. In the operated embryo the liver pushes over to the left, instead of to the right, in the earliest stages observed, although its material lay far below the level of the operation. The dorsal and ventral pancreas unite, not on the right side, but on the left.

In all Pressler's nineteen cases, where situs inversus viscerum is present, the heart is inverted and this can be traced to its earliest stages. The right yolk-vein is larger than the left, and the heart bends to the left instead of to the right. Its spiral turns anticlockwise. The inversion, that is present, involves even the finest details of structure, and, since the heart lies beyond the field of operation, its inversion must be supposed to be induced by changes that first occur in the region of the liver. In consequence, one of the yolk-veins is smaller than the other, and Pressler, following Spemann, suggested that the change in the direction of the blood-flow may be the immediate cause of the inversion of the heart.

Several years later ('13) another student, Meyer, working with Spemann, made a larger number of operations of the same kind. He examined in more detail the very early stages of the normal asymmetry, and has made out a few further significant relations. His general conclusions also differ to some extent from those of Spemann and of Pressler. Meyer obtained eight cases of inversion (out of 9 operations) in Bombinator; 16 cases (out of 19) of Rana esculenta; and 6 of Bufo.

A study of the normal conditions in young embryos at a time when the first asymmetry could be detected showed that at the stage when the liver begins to turn a little to the right, the visceral

sheet of mesoderm on each side of the endothelial tube of the heart shows also a slight asymmetry (Fig. 72*c*). On the right side there is a slight bend in the visceral sheet, while on the left side it takes a straighter course. The slight bend in the right side corresponds with the later bending of the S-shaped tube and beyond doubt is the first indication of future relations.

In these earliest stages the yolk-veins have scarcely formed and are not yet hollow vessels carrying the blood as they are a little later, and since at this stage there is no blood circulation, it appears improbable that the bending of the heart tube can primarily be due to the greater flow of blood from one of the yolk-veins.

Meyer found in the operated embryos (with an inverted neural plate) that the liver turns to the left, and the relation of the right and left visceral layers is the reverse of that of the normal embryo. These observations show that here also the asymmetry is initiated very early, and may be due, to the position of the liver. This, in turn, affects the visceral walls of mesoderm that extend over it and then forward to the heart region. The inference seems reasonable that the differences seen in the two visceral layers, right and left, are initiated by the direction taken by the liver outgrowth.

Since at the time of the operation the mesoderm also extends under the neural plate and is inverted with the plate, it might appear possible that the mesoderm of the heart region might also be affected indirectly by this inversion. Such a view is rendered improbable by the fact that the dorsal inverted mesoderm lies behind the level of the future heart region, i.e., it is not mesoderm lying on the dorsal side above the heart level.

Meyer attempted to find out by a critical experiment whether situs inversus is due to the inversion of the whole dorsal piece or only to the inversion of the dorsal wall of the archenteron. A square cut was made, and the neural plate (ectoderm) and the underlying mesoderm were lifted off, leaving in place the dorsal wall of the archenteron. It was found impossible to leave the endoderm intact, but in some cases a large part of it (more or less torn) remained. The plate removed was then inverted and replanted in the opening. In five cases, both digestive tract and heart were found to be inverted, possibly because enough endoderm had been left sticking to the inverted plate to cause inversion

in the digestive tract. In three cases, on the other hand, no inversion took place, either in the digestive tract, or in the heart, because, possibly, enough endoderm had been left to maintain the normal relations. The last result shows at least that the inversion is probably not caused by the action of the neural plate. It may be added that both Spemann and Meyer found that if the square plate after being cut out was not replaced, but the opening was allowed to close, no inversion took place.

The influence of the inversion on the position of the spiraculum was also examined by Meyer. In Bombinator it is ventral and median; in Rana and Bufo it lies on the left side. Spemann found that, in one case, the spiraculum was inverted also, i.e., it lay on the right side. In two other cases it did not unite across the middle line. Meyer found in five cases of situs inversus in Rana esculenta that the spiraculum opened on the other side (Fig. 71*d*). This occurred also in one larva of Bufo.

Fig. 73.—A double-headed embryo of Triton with situs inversus of the digestive tract of one embryo which developed from a constricted gastrula. (After Spemann.)

There were a few cases in which the digestive tract was inverted, but not the heart. Such cases may possibly be due to the normal position of the heart having been already determined before the time of operation. If so, the inverted plate acted on the digestive tract, and the heart continued to develop in response to the earlier influence of the normal relations.

In this connection some experiments of a very different kind should be described that also bring about inversion of the digestive tract and heart. These results were obtained first by Spemann ('01, '03) and later by Spemann and Falkenberg ('19).

When a ligature is placed around the egg of Triton in the plane of the first cleavage in an egg in which the first plane coincides with the future median plane, an embryo with a double

anterior end develops (Fig. 73). If the constricting thread had been gradually tightened, until the two halves were completely separated, two embryos developed, one from the right and other from the left half (Fig. 74*a*, *b*). These embryos were often defective especially on the "inner" side, but the results in regard to inversion seem to bear no direct relation to the extent of the abnormality. Of 25 sinistral larvae both the heart and the digestive tract had a normal orientation. Only in one was the heart inverted while the condition of the digestive tract was uncer-

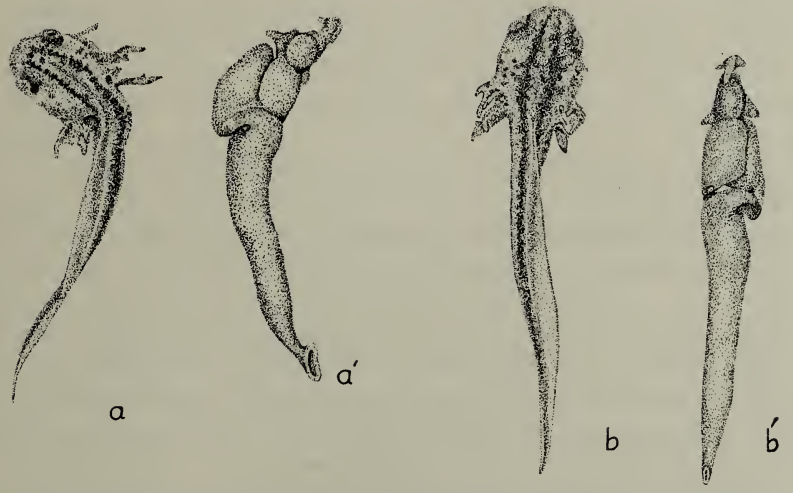


FIG. 74.—*a* and *b*, left and right embryos from an egg constricted at the first cleavage in the prospective median plane into two separate parts; *a′* and *b′*, the digestive tract of the same two embryos showing situs inversus viscerum of one embryo. (After Spemann.)

tain. Of 30 dextral larvae the inversion of the heart and digestive tract was clearly present in 12 embryos, and probably in 2 others; the rest were normal.

Two other pairs may be added to the above list, in both of which the sinistral embryo is normal, the dextral inverted. Other cases in which the halves remain partly united show sometimes the same relations. In 12 cases the left component was normal; the right, anterior region of the digestive tract was normal in only two cases; in the remaining ten embryos the anterior part was inverted.[1]

[1] Mangold ('21) has shown that situs inversus occurs normally, i.e., without operation, in about 2 per cent of the larvæ of Triton taeniatus and in nearly

Spemann was inclined to refer the asymmetries of the normal embryo either to a primary micro-structure of the egg, or to some asymmetrical action of the sperm on the egg. But even on such an assumption it must be conceded that this "fundamental" structure can be readily inverted, as shown by the experiments. Since the double embryos are, when inversion occurs, mirror figures of each other, and since this relation is not an uncommon accompaniment of paired whole structures, it might seem promising to refer the result to this general category, even although there is at present no adequate causal explanation of the mirror-figure relation. But in the separated embryos the mirroring takes place when the parts are entirely separated. I am inclined to look, therefore, to some secondary condition imposed on one of the two embryos as a result of the deficiency on their inner side, a condition that supplements the initial steps, already present in the left embryo, but which acts inversely on the conditions of the right embryo. For example, if the digestive tract and liver of the left embryo is displaced somewhat to the right (the inner or defective side) such a change is in the direction taken by the liver in the normal embryo and no inversion is expected. On the other hand, a similar shift in the right embryo will displace the digestive tract and liver to the left (the inner or defective side) and such a change is in the opposite direction to that taken by the liver in the normal embryo, and inversion might result.

The preceding cases, in which two embryos were obtained from a single egg, recall the double embryos (double monsters) that sometimes are found on the same egg. This is not an uncommon occurrence in fish-embryos. In some of these cases it has been found, in fact, that the heart and digestive tract are reversed in one of the embryos (Morrill '19, Swett '21). How far this result is due to the degree of separation of the two embryos, and how far to a mutual influence of one on the other of such a sort that they become mirror figures of each other, will be considered in another connection.

The asymmetry of the aortic arch in birds and in mammals

the same ratio in T. alpestris. When, in these, the heart is inverted the intestine is always inverted, but the liver or the intestine may be inverted and not the heart. Wilhelmi ('21) brought about inversion by removing a part of the left posterior side of an embryo when the neural fold was about to develop (one case in five).

is another instance of asymmetry superimposed on a fundamental symmetry. The suppression of the right ovary and oviduct in birds may be connected with the turning of the embryo onto its left side at an early stage, but this relation has not been established, although it might possibly be tested in those not infrequent cases where the chick lies on its right side. Dareste ('77) produced situs inversus in chicks by heating the left side of the egg (embryo) more than the right. The result has been later confirmed by Warynsky and Fol ('84); but the evidence is too meagre to throw much light on the situation. Recently Congdon and Wang ('26) have described the changes that take place in the aortic arches of birds and mammals that lead to the degeneration of some of the arches of one side, but the initial steps remain unaccounted for.

Similar questions concerning the origin of symmetry in limb-buds of amphibia grafted into other parts of the embryo and in reversed relations, and the minor symmetries of regenerated parts and of identical twins will be considered in other connections.

The Origin of Asymmetry in Serpulids and Fiddler Crabs

Some of the sedentary annelids, such as Serpula and Hydroides, have a sort of plug (operculum) that closes the tube when the worm retreats into it. The plug is attached to one side of the middle line (Fig. 75*d*, *e*). On the opposite side there is a tiny, rudimentary plug. Both of these represent modified gills of the young embryo (Fig. 75*a*). Zeleny ('01) has shown that if the large plug of the adult worm is cut off, the smaller one begins to grow, and in the course of a few weeks becomes as large as the one removed, and acts as the functional plug. From the stump of the original large plug a new, small plug develops. If now this secondary functioning plug is removed, the secondary small one enlarges to full size, and from the stump a small one develops, etc. This evidence indicates that the presence of one functional operculum holds its mate in check. The nature of this influence is not known. One is tempted to refer it to some material produced by the more developed plug that holds the other in check, or by something used up by the larger plug that the smaller one needs for development. Both of these views may, however, seem self-contradictory, since the influence imagined would

be supposed to act, at first in both alike unless a threshold value is assumed. Since either the right or the left may be the functional plug, it would then appear to be only a question of chance which one got the start of the other. Such an interpretation would call for no contrivance in the egg to throw the balance to one side or the other.

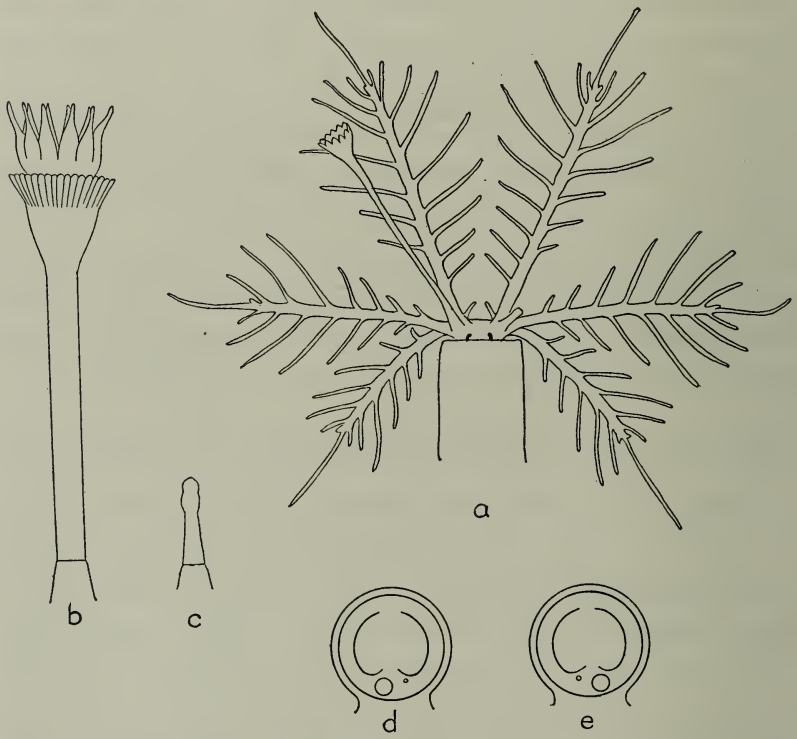
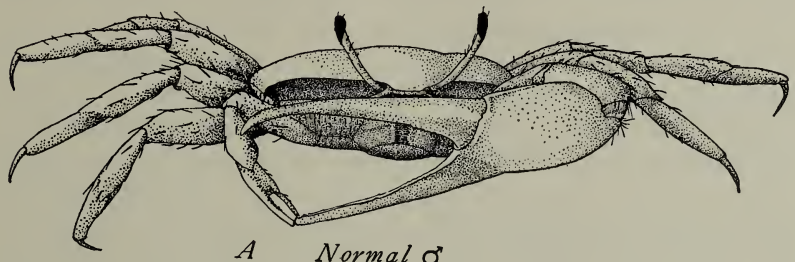


FIG. 75.—*a*, young stage or Hydroides, showing one branch of the gill modified as an operculum; *b* and *c*, functional and rudimentary opercula of adult; *d* and *e*, diagrams showing the attachment of the opercula. (After Zeleny.)

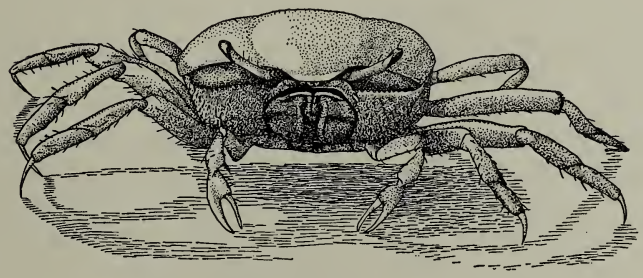
The first pair of legs bearing the large "claws," of many crabs, lobsters and other crustacea are different on the two sides and markedly so in the male fiddler crab (Fig. 76) and in Alpheus. Here the larger claw may be either the right or the left one. There is no predetermined asymmetry. Once determined, however, it may be made to reverse in some species but not in others. As shown by Przibram ('00) removal of the large claw of Alpheus leads, at the next molt, to its substitution by the smaller claw on

the other side; but in other decapods, as in the fiddler crab, the large claw of the adult, if removed, develops again, and is not substituted for by the claw on the other side.

The development of asymmetry in the male fiddler crab (Gelasimus) has recently been worked out by Morgan ('20, '23,



A *Normal* ♂



B *Normal* ♀

Fig. 76.—*A* and *B*, adult male and female fiddler crab, showing sexual dimorphism. (After Morgan.)

Fig. 77.—*A*, young male crab at an early stage when both claws have more the form of the male claw; *B*, young crab that has lost the left claw and has regenerated a new claw on the right side like that of the female. (After Morgan.)

'24). In the young stages of the male the two claws are present, and both are a little larger than those of the female of the same age and show the swollen shape of the male "large" claw (Fig. 77*A*). At about this time most of the young crabs lose one or the other of these "large" claws and produce at the next molt,

in place of the one lost, a small claw like the small male claw or like the two small claws of the female (Fig. 76*B*). This relation, once attained, becomes fixed, and even in young crabs no reversal after the removal of the large claw is possible. An accident, then, determines in this crab the first asymmetry of the claws of the male. There seems to be nothing in the egg, or in the development that brings about the asymmetry.

If the young male with his large claws is isolated, and carefully protected from injury and fed, he usually retains both claws throughout successive moults and develops into a symmetrical male with two large claws. There are only two records of such adults found in nature, and since this occurrence is extremely rare, it might be expected that the loss of one claw, even at a somewhat later stage than that at which the loss normally occurs, is the event that generally leads to the asymmetry of the adult. In fact, experiments with very young males, that have retained both claws through two or more moults later than the moult at which the loss usually occurs, show that a small claw develops in place of the large one removed.

The converse experiment has also been carried out. Both claws were removed from young males with two large claws. At the next moult two small claws developed and in the two or three subsequent moults both claws remained small. If this condition persists into the adult stage, a symmetrical male would develop with two small claws. A few such adult males have, in fact, been found (Morgan '23).

In a few cases where two secondary small claws were induced, as just described, one small claw was then removed. A small claw came back, and the opposite claw also remained small. The induced symmetry has inhibited the individual from acquiring the normal asymmetry. In other words, when at the critical stage the individual has been led to take a false step, no subsequent rectification is possible.

Here, then, asymmetry is introduced, as a rule, relatively late in development. There is nothing in the egg responsible for the asymmetry unless it be the reaction-system that responds at a certain stage in the development to an induced asymmetry. If, in the young male crabs, the right claw is lost as often as the left we can understand how it comes about that half the adult males have the right claw large and half have the left claw.

Reversals of Symmetry in Echinoderms and in Ascaris

A few instances of situs inversus have been observed in larval sea-urchins (Runnström '12, Oshima '22) and in starfishes (Müller '46, Masterman '02, Mortensen '21). The pleuteus stage of sea-urchins is normally asymmetrical; a functional hydrocoel being present only on the left side. In the reversed pleuteus it lies on the right side. Several instances have also been described both in larval sea-urchins and in starfish (Newman '23) where both a left and a right hydrocoel are present. Such larvae are symmetrical, standing, as it were, midway between the normal and the reversed condition.

The thread worm of the horse, whose eggs have a determinate and symmetrical type of cleavage, shows distinct asymmetries in the nervous system and in the excretory vessels of the adult, which are typically on the same side. Zur Strassen ('96) found that about one egg in 40 has "inverse" cleavage, and that 4 adults out of 125 showed reversal. It seems plausible, as he points out, to assume that the reversed cleavage type gives rise to the reversed adult type. On the other hand, Bonfig ('25) has suggested that the result may be connected with antero-posterior reversal of the rhomboid stage, that has been described in connection with the development of symmetry of these worms.

The Asymmetry of Snails

In gasteropod molluscs the visceral mass on which the shell is molded is twisted into a right- (Fig. 78*c*') or a left-handed spiral (Fig. 78*c*). In some species the spiral is typically dextral (Limnaea and Crepidula); in other species it is sinistral (Physa and Planorbis). Even within the same species there may be dextral and sinistral individuals. For example, some species of the Achatinellidae are typically right-wound (dextral); others left-wound (sinistral), but in some species both dextral and sinistral snails are found (Mayer '02, Gulick '05). Similar conditions are also found in Tahitian snails (Crampton '16). In several dextral land snails of Europe, sinistral individuals have been frequently described (Lang '04, Hesse '14), and in certain localities reversed forms of several fresh water snails have been recorded (Dewitz '16, Boycott and Diver '23).

Crampton ('94) pointed out that the spiral type of cleavage is reversed in dextral and sinistral snails (Figs. 78a^1, b^1, a, b). In consequence of this relation, the mesoderm cell, 4d (Fig. 79)

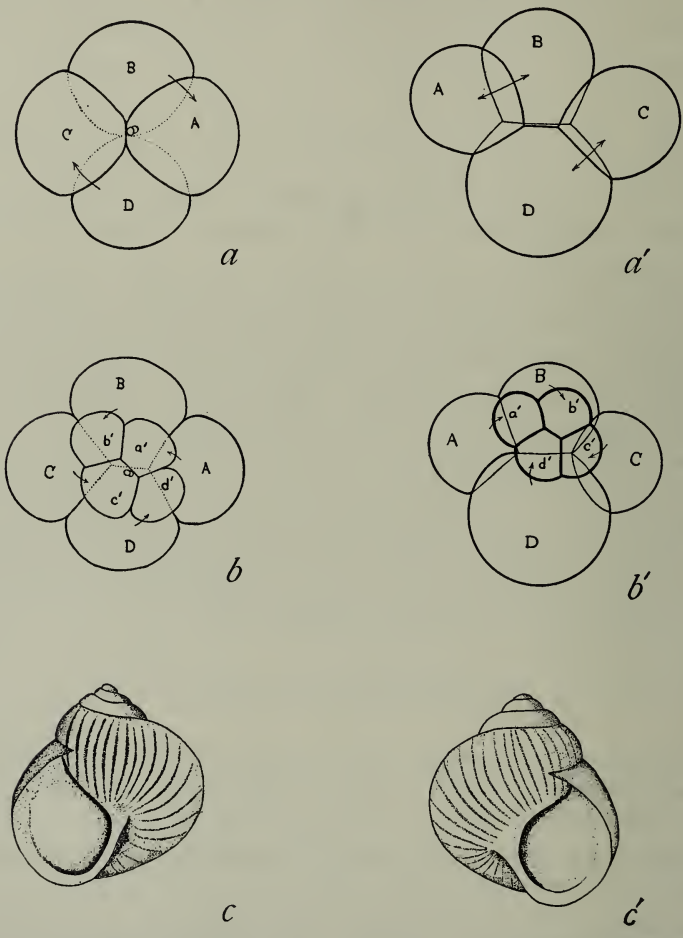


FIG. 78.—*a*, *b*, and *c*, left-handed type of cleavage and left wound spiral shell of mollusc; *a′*, *b′*, and *c′*, right-handed type of cleavage and right wound spiral shell of mollusc. (After Conklin)

that is generally larger than the other members of the fourth quartet (4a, 4b, 4c), lies to the right of the first plane of cleavage in one type and to the left in the other (Fig. 79*a*, *b*).

The asymmetry has been traced to the position of the spindles for the second division of the egg (Crampton) where its direction

is diagnosed by the "cross-furrow" at the meeting point of two opposite blastomeres as seen in the polar hemisphere. As shown

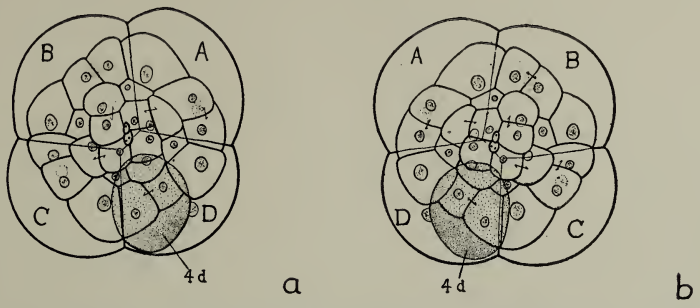


Fig. 79.—*a*, later cleavage stage of egg of mollusc in which the 4d cell has been given off on the right side; *b*, same with 4d on the left side.

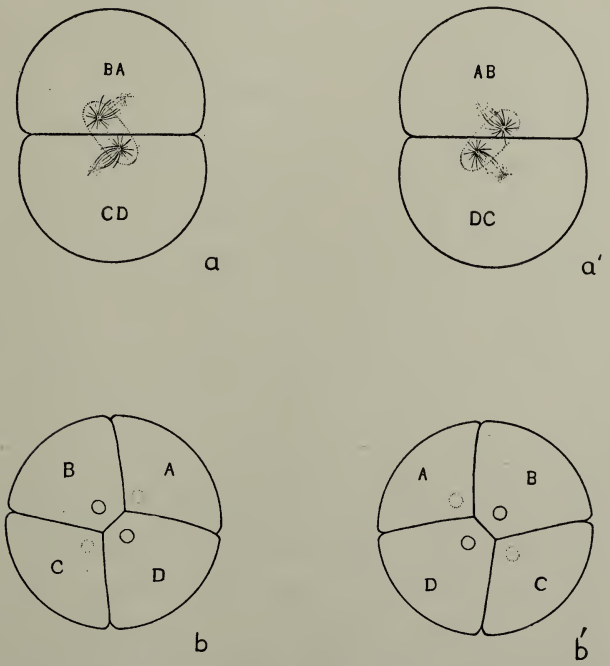


Fig. 80.—*a*, first cleavage stage showing the position of the spindles for the next cleavage in a left-handed type of cleavage; *b*, same after cleavage, antipolar view as seen from above; *a'*, like *a* in right-handed type; *b'*, antipolar view as seen from above.

in Figs. 78*a* and a^1 the cross-furrow in the sinistral type (*a*) is the mirror figure of that in the dextral type (a^1). In the dextral type (Fig. 80a^1) the right end of the spindle in the AB blastomere

is higher than the left end of the same spindle; and vice versa for the spindle in the DC blastomere. Since later cleavages alternate between dextral and sinistral, Conklin ('05) suggests that even the first spindle may be dextral in the dextral type and sinistral in the sinistral type of cleavage. This has, however, not been demonstrated, although it seems quite probable.

At the third division of the egg four small cells are pinched off around the pole of the egg by a clockwise division in the dextral type (Fig. 78*b*[1]), and by an anti-clockwise division in the sinistral type (Fig. 78*b*). After this division, quartettes of small cells are formed from the four large cells (ABCD) by alternate anti-clockwise and clockwise divisions. This continues until, at the seventh division, a large cell (4*d*) is pinched off from the large D cell (Fig. 79*b*). This cell, as stated above, is the first asymmetrical division in the sense that this cell (4*d*) is sometimes given off sooner and is often much larger than the corresponding cells of the other quartets. The 4d cell lies on the posterior side of the embryo, and in the dextral type to the left of the middle line (Fig. 79) which corresponds nearly to the second plane of cleavage. In the sinistral type the 4d cell lies to the right of the middle line (Fig. 79*a*). The asymmetrical position of this cell, from which the middle layer of the embryo arises, suggests that the later asymmetry of the body is built up on this relation. On the other hand, the position of 4d may only be an expression of the type of cleavage, and the latter rather than 4d itself, may be the forerunner of the asymmetrical structure that develops.

Several attempts have been made to explain the spiral type of cleavage of the molluscan egg as well as the origin of the reversals seen in certain cases. Rabl ('00) called attention to spiral asters in certain eggs described by Mark ('81) and by Kostanecki and Wierzejsky ('96). He thought these spirals might indicate a spiral foundation in the cytoplasm responsible for the spiral cleavage, but later research has not confirmed these earlier observations as typical. In fact, the spiral aster is probably an artifact (Byrnes '99).

Conklin ('03) pointed out that, if, in snails having the right-handed spiral type, the polar bodies are formed at the pole of the egg and the quartets are formed about the same pole, and if in the left-handed species the polar bodies are given off at the

antipole, and the quartets are also formed at the antipole, then the two types would show reversed spirals if these depended on the same fundamental spiral structure in the cytoplasm. Conklin has himself called attention to the difficulties in making such an interpretation, and there is no evidence that the poles are reversed in the two types of eggs, and much evidence that they are not.

Holmes ('00) suggested that if the first cleavage of the dextral form corresponds to the second cleavage of the sinistral types this would account for the reversal of the spirals. At present there is no evidence of such substitution. The only advantage that such a view might offer would be that the spiral structure of the protoplasm, if such exists, might then be assumed to be the same in both forms. It may seem hazardous, in the light of these failures, to attempt at present to suggest a solution of the two types of spiral cleavages leading to the final asymmetry of the snail, were it not that in one respect at least we have recently made a step forward. If, as the genetic evidence has made probable, the twisting is inherited as a Mendelian character, and is possibly a special case of maternal inheritance, then the character may be impressed on the ovarian egg before the polar bodies are extruded. Two possibilities are then given: either the influence is exerted from the egg-nucleus before its maturation divisions, or else it is determined by the follicle cells of the mother that surround the egg during its growth. The former alternative offers no further clue as to how the cytoplasm is affected; the latter alternative may appear at first sight to offer a suggestion. For example, if the polar region of the egg were supposed to be oval in shape (as a result of the influence of the follicle), and if in the dextral type the oval is turned to the right (Fig. 80a^1) and in the sinistral type to the left (Fig. 80a) and if the position of the two spindles at the second division is determined by this polar field, then it might appear that the differences in the two types could be accounted for.

On the other hand, there are obvious objections to this interpretation. It would have to be supposed, for instance, that in a snail that is actually sinistral (owing to its maternal inheritance) but genetically dextral, the latter influence, and not the form of the twist, determines the character of the egg. In the second place, it has been shown by Wilson ('04) in the mollusc, Dentalium, whose egg cleaves spirally, that fragments both of the

upper and of the lower hemisphere cleave spirally in the same way as does the whole egg. Such a result is inconsistent with the assumption that the spiral is due to an influence starting during cleavage from the polar field. It might also be urged that the dextral type of cleavage is present in forms like Nereis that are themselves bilateral; but this is not, perhaps, a serious objection, since it is not the shape of the individual that determines the twist, by hypothesis, but the genetic make-up of its cells.

Under these circumstances it is apparent that the hypothesis suggested above is entirely inadequate. The situation, as it appears at present, may possibly be stated as follows:

(1) The genetic evidence indicates that the type of cleavage, dextral or sinistral, is inherited probably as a Mendelian pair of characters. Furthermore, the inheritance appears to belong to that category of cases known as maternal inheritance.

(2) Since the characteristic type of cleavage is shown by fragments taken from any region of the egg, it is not probable that it can be traced to any localized structure on or in the egg, but is a peculiarity that develops in the protoplasm at about the time of fertilization and need not be supposed to be present as such before this time.

(3) It is not necessary to assume that this peculiarity is expressed as a spiral structure in the protoplasm. There may well be other influences that turn the first spindle in an oblique position with respect to the primary axis and at the same time direct the spindle in a clockwise (or anti-clockwise) direction. The later reversals of the cleavage may be due to the orderly sequence of the successive spindles at right angles to the last ones (or possibly also to the form of the preceding cells). This view avoids the difficulty of assuming that the influence that gives the first spiral reverses itself at each subsequent division.

We need at present critical experiments to test these and other possibilities. By compressing the egg, by studying the cleavage of fragments of sinistral types, or by altering the conditions of the egg in other ways, it should be possible to discover the nature of the protoplasmic factors that determine the spiral cleavage pattern.

Finally, since the position of the first cleavage plane is not predetermined, either in the bilateral or in the spiral types,

it follows that the median plane of the embryo may coincide with any meridian of the egg. Therefore, whatever the nature of the conditions that lead to a spiral cleavage they carry with them the condition that locates at the same time the bilaterality of the embryo. The two may possibly be regarded as part of the same process.

It was shown by Crampton and by Kofoid ('94) that these two types can be distinguished as early as the second cleavage, and perhaps even at the first, by the form of the cleavage pattern —one is a mirror figure of the other. It was known from the observations of Mayor ('02) and of Crampton ('17) on Partula, and has been confirmed by the recent work of Boycott and Diver ('23) on Lymnaea, that all the offspring of a given brood are dextral or else sinistral. It has also been shown that some sinistral mothers produce only sinistral broods and that other sinistral mothers produce dextral broods. Conversely, some dextral mothers may produce only sinistral broods and other dextral mothers may produce dextral broods. These facts were very puzzling from a genetic standpoint, and there was no satisfactory explanation at hand. But recent experiments of Diver, Boycott and Garstang ('25) have supplied data which Sturtevant ('23) has shown can be interpreted, if the character of the cleavage (hence the character of the adult) is impressed on the egg by its genetic make-up before the maturation divisions have occurred. An example will serve to show the principle involved.

Suppose, as the evidence indicates, there is a dominant dextral and a recessive sinistral factor carried by a given pair of chromosomes. A self-fertilizing dextral snail that is heterozygous for these factors (Ll) produces after maturation two kinds of eggs L and l. Similarly, there will be two kinds of sperm; namely, L and l. Self-fertilization will give three genetic types of offspring—LL, Ll and ll; but all these individuals will be dextral because the cleavage pattern has been already determined in the egg by the dominant factor L before the polar bodies were given off. Of these three types the first two LL and Ll will produce only dextral offspring, but the other type ll, that has also a dextral shell will produce only sinistral offspring. Since these snails may also cross-fertilize, provided dextral mates to dextral, and sinistral to sinistral, it is possible for the dextral

female (arising as above) with the genetic constitution ll to mate with a dextral with the composition LL. All the offspring of such a somatically dextral female will be sinistral, since the undivided egg was under the influence of the two recessive genes (ll). These sinistral snails (Ll) in turn will produce only dextral offspring because the dominant factor L in the egg determines the type of cleavage of the eggs. It is evident, therefore, that dextrals of certain origins will produce only sinistral broods and sinistrals of certain origins dextral broods. The heredity is Mendelian, but the appearance of the character is delayed for a generation. The result is unique, because the symmetry of the adult is determined not by its own genetic constitution but by that of the unreduced egg from which it arose. There is no contradiction in this to ordinary Mendelian inheritance of adult character, if as appears to be true, the symmetry is determined by the constitution of the egg before extrusion of the polar bodies, and, once determined, cannot later be reversed, no matter what the genetic constitution of the zygote has become.

Sturtevant's hypothesis to account for the *inheritance* of dextrality and sinistrality is the simplest that has been proposed. It covers the main facts, but recognizes that there are exceptional cases that it does not pretend to explain. Whether these exceptions are as significant as Diver ('25) believes, or whether they will be traced to modifying influences, or be due to occasional mutations remains to be determined, since critical experiments have not as yet been carried out.

In a later paper Diver states that he and Boycott "did not feel justified in putting forward the very attractive interpretation since proposed by Sturtevant" because of the 3:1 ratio obtained in certain broods, and the absence of "compensating" broods in the supposedly 1:3 ratios (see below). In so far as the earlier data referred to were based on the progeny of pairs kept together, the evidence has little value; for any ratio may be expected under these circumstances from any hermaphroditic pair. Whether there should be a "compensation" between the 3:1 and the 1:3 broods, etc., may depend on viability and other possible relations. It is interesting to observe that in the later paper (Diver '25) the essential part of Sturtevant's hypothesis (viz., maternal inheritance) is accepted, but the Mendelian character of the results is nullified by peculiar assumptions regarding the absence of

dominance in the presence of a strictly alternate type of inheritance.[2]

The following list gives the kinds of broods that have been obtained:

A	B	C
All dextral	3 dextral to 1 sinistral	1 dextral to 1 sinistral
D	**E**	**F**
All sinistral	Mostly sinistral with a few odd dextrals	Mostly dextral with a few odd sinistrals

Diver concludes from the evidence that except for the behavior of dextrality in a "mixed" brood (type B) "there appears to be no evidence of dominance," but this of course is not expected from a record of broods but must be discovered, if present, in the kinds of individuals that appear from some of the individuals of some of these broods in later generations. On the other hand, if, as he has found, a single isolated sinistral individual can give an all sinistral brood containing some individuals which again by self-fertilization will in the next generation give an all dextral brood (type A), then it must be admitted that occasionally something happens that is beyond the expectation of maternal inheritance and dominance of dextrality. Whether these exceptions can be best met by Diver's dubious view of no dominance, or by a high mutability in the allelomorphic genes that stand for one or the other type, or by an environmental effect, can only be determined when critical evidence is forthcoming.

[2] The particular assumption referred to here, that diverges from Mendelian convention, may be exemplified by Diver's statement that when a dextral determiner and a sinistral determiner (chromosome) meet "both should theoretically exert an equal and opposite force, and the resultant would be zero, i.e., a shall and an animal that are bilaterally symmetrical, which in this case is presumably an impossible condition. Practically, the probability would be that in half of these cases one force, and in the other half the other force, would be effectively the stronger, and this has been expressed by saying that half the heterogeneous nuclei will give an appearance—determiner—R L, and half L R, thus:

$$\overset{((}{RR}\text{(dextral).}\quad \overset{()}{RL}\text{ (dextral).}\quad \overset{)(}{LR}\text{(sinistral).}\quad \overset{))}{LL}\text{(sinistral).}$$

CHAPTER XV

LOCALIZATION BEFORE CLEAVAGE: THE DEVELOPMENT OF EGG-FRAGMENTS

In order to find out whether the different regions of the unsegmented egg bear any definite relation to subsequent events in the development, the egg has been broken into fragments, or pieces have been cut off, both before and after fertilization, and the development of the fragments has been followed.

In those eggs in which the large egg-nucleus is present when the egg is removed from the female, and in which the extrusion of the polar bodies takes place after its removal (Cerebratulus) there is given an opportunity of finding out whether, during this period, alterations are taking place that condition the cleavage pattern. In these same eggs, as well as in those in which the polar bodies have been already extruded when they are obtained (sea-urchins), there is an opportunity of finding out how far the cleavage pattern is determined after the polar bodies are given off and before or after the entrance of the spermatozoön into the egg. The study of fragments has also shown to what extent the cleavage pattern is foreshadowed as the time approaches when the first division is about to take place.

The earliest observations on egg-fragments were made on pieces of the sea-urchin's egg (Hertwig '87, Driesch '92, Morgan '95, Delage '99, Stevens '02, Boveri '89, '96, '14, '18). The fragments were obtained in most cases by violently shaking eggs until they were fragmented. This method is not satisfactory, both because the position of the fragment in the egg from which it came is not known, and because in the sea-urchin the observations are limited to the period after the polar bodies have been formed. More significant results have come from the study of fragments, that have been cut from the egg along known planes. Operations of this kind have been made on the eggs of Cerebratulus (Wilson '03; Zeleny '04; Yatsu '04); of Dentalium (Delage '99;

Wilson '04); and of Beroë, (Driesch and Morgan '95; Yatsu '10, '12; Fischel '98, '03); and of the sea-urchin (Stevens '02; Taylor and Tennent '24; Harnly '26; Taylor and Whitaker '25, '26). A somewhat similar method consists in constricting the egg of Triton by a hair loop or thread (Spemann '14; Baltzer '20, '22; Fankhauser '24, '25).

The Devleopment of Fragments of the Egg of the Nemertean Cerebratulus

Normal eggs are obtained in abundance by cutting open the body of the worm. The eggs are at first somewhat irregular in outline, but very quickly round out into spherical shape. Over the egg there is a gelatinous envelope drawn out into a pointed protuberance (Fig. 81*a*) at the antipole. This protuberance has,

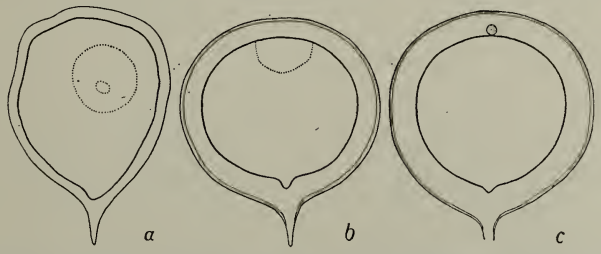


Fig. 81.—Egg of Cerebratulus during the ripening stages. In *a*, the egg-nucleus (germinal vesicle) is still present. In *b*, the polar spindle has formed, and in *c*, the first polar body has been given off. (After Wilson.)

at first, a core of protoplasm that extends to the point of attachment of the egg to the wall of the ovary, and serves as a landmark during the maturation of the egg. The nucleus is large, when the egg is removed from the worm, but almost immediately fades out (whether the egg is fertilized or not), and the first polar spindle develops (Fig. 81*b*). The spindle moves to the pole of the egg, and remains in the metaphase until fertilization takes place. Two polar bodies are then produced in succession (Fig. 81*c*), and the ordinary process of union of the two pronuclei and the formation of the segmentation spindle follow.

The egg divides into equal parts, the plane of division passing through the pole (Fig. 82*a*). The second cleavage is at right angles to the first, and also passes through the pole producing

four equal cells (Fig. 82*b*). There are no marked cross-furrows at either pole. The third cleavage may be described as nearly equatorial. It is slightly dexiotropic (Fig. 82*c*). The four cells around the pole are *larger* than the four cells around the antipole. The larger size of the four polar ("upper") cells is "unique in the spiral type of cleavage." From this time onward the divisions are alternately leiotropic and dexiotropic (Fig. 82*d*). Later the antipolar region flattens and invaginates to form a gastrula. The larva (Fig. 86*a*) is called a pilidium.

The development of egg-fragments of Cerebratulus was first studied by E. B. Wilson. The results have been confirmed and

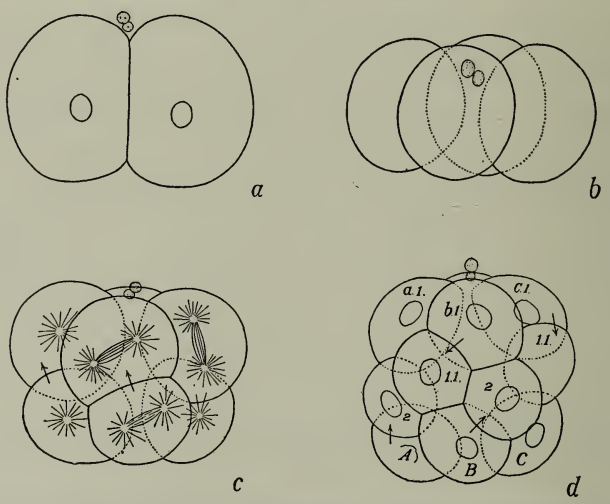


Fig. 82.—Normal cleavage of Cerebratulus. (After Zeleny and Wilson.)

extended by the work of Zeleny and Yatsu. A general account will first be given and a more detailed one afterward.

After the dissolution of the germinal vesicle (Fig. 83) the egg may be cut in two with a scalpel in any desired plane. Both fragments remain intact, and soon round up to become spherical or nearly spherical. One fragment the polar one, contains the egg-nucleus, the other, the antipolar one, is without a nucleus. The nucleus of the former proceeds to resolve itself into the first polar spindle. When the metaphase stage is reached, further progress stops until a spermatozoön enters the fragment. In this respect the nucleated fragment behaves in the same way as an intact egg. The subsequent history of this fragment is as

follows: Its cleavage pattern (Fig. 84) is the same as that of the whole egg. The blastomeres have the same relative proportions as have those of the normal egg. For example at the third division of the normal egg (Fig. 82*c*) the spiral type of cleavage appears; the fragments also divide at this time in a right-handed spiral (Fig. 84*c*, *d*). The result is the same, regardless of the region of the egg from which the fragment comes. In some cases, as shown in Fig. 84*c*, the cut was "horizontal" and in other cases "vertical" (Fig. 84*a*) and in others "oblique"; nevertheless, the cleavage pattern is the same in all cases. This result shows that

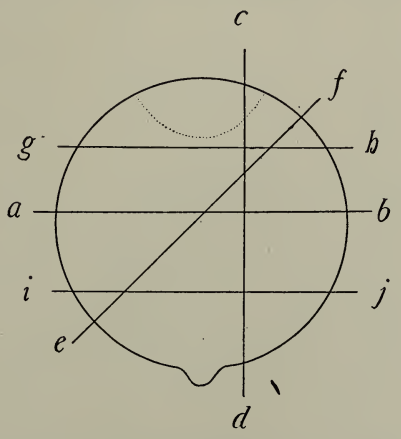


Fig. 83.—Egg of Cerebratulus showing the planes of cutting. (After Wilson.)

prior to the breaking down of the egg-nucleus there are no regional differences present in the egg that determine the form of its cleavage. From these fragments, if not very small, normal embryos develop that are miniature copies of the whole embryo (Fig. 86*b-d*).

The other piece of the egg, the one without a nucleus, can also be fertilized by a spermatozoön. It, too, shows the normal cleavage pattern (Fig. 85*c*, *e*). Since its nucleus is derived solely from the head of the spermatozoön, the fragment contains only half the full number of chromosomes (haploid). This condition, however, does not affect its mode of cleavage. It is true that the haploid nature of the fragment has not been proven in this case, but since its cleavage is said to be synchronous with that of the other piece, there can be little doubt as to its condition. This

haploid fragment also forms a normal embryo, if not too small, irrespective of the region from which the fragment was removed (Fig. 86).

If instead of fertilizing the fragments as soon as obtained the operation is delayed until the first polar spindle has developed in the nucleated fragment, the results are the same as before.

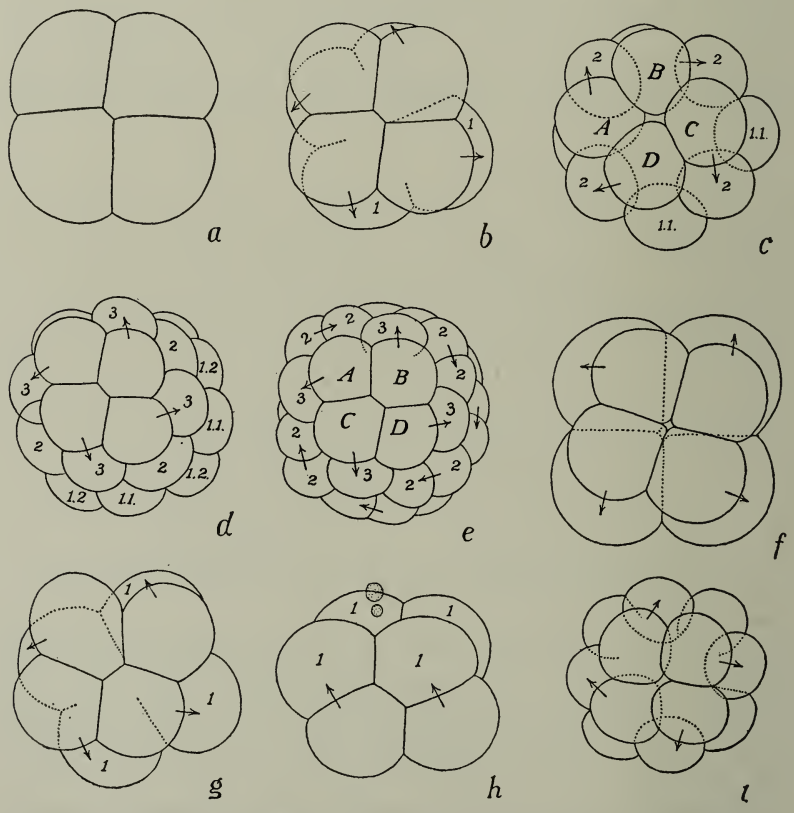


Fig. 84.—Cleavage of fragments of the egg of Cerebratulus. (After Wilson.)

This evidence shows that the cleavage of the fragments is typically a whole cleavage of reduced size without respect to the part of the egg from which the fragment is derived. Further details concerning the cleavage of different fragments are given in Figs. 84 and 85. A typical cleavage of an antipolar fragment ("horizontal section") is drawn in Fig. 84*a-e*, as seen from the antipole. An eight-cell stage of a polar fragment (sec-

tion *i-j*, Fig. 83), as seen from the antipole, is drawn in Fig. 84*f*. This antipolar quartette is proportionately smaller then in the normal cleavage. Two eight-cell stages of two fragments of the same egg are drawn in Fig. 84*g*, and *h* (oblique sections *e-f*, Fig. 83). A typical sixteen-cell stage of the non-nucleated fragment of the same egg is drawn from the "lower" pole in Fig. 84*i*.

The cleavage of another series of fragments is shown in Fig. 85. Two typical four-cell stages of two fragments of the same egg (vertical sections *c-d*, Fig. 83) are drawn in *a* and *b*, Fig. 85. An eight-cell stage of a nucleated fragment from the "upper" hemisphere is drawn in Fig. 85*c*. The non-nucleated sister frag-

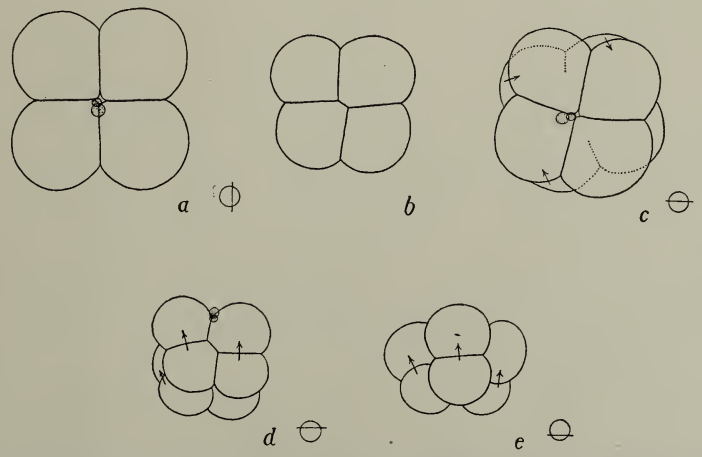


FIG. 85.—Cleavage of fragments of the egg of Cerebratulus. The small circles to the right of each figure, with the line running through them, show the plane of cutting. (After Wilson.)

ment divided in the same way. A typical eight-cell stage of a small fragment of the "polar" hemisphere (horizontal section *g-h*, Fig. 83), is drawn in Fig. 85*d*. A nearly typical eight-cell stage from the "antipolar" hemisphere (horizontal section *i-j*, Fig. 83) is drawn in Fig. 85*e*.

Some of the pilidia that developed from fragments are shown in Fig. 86*b*, *c*, *d*. Of these, Fig. 86*b*, is from the "polar" hemisphere and is not quite normal; *d* is also from the "polar" hemisphere and is normal; *c* is from the "antipolar" hemisphere. For comparison, a whole pilidium is drawn in Fig. 86*a*.

"These observations prove that in the eggs, prior to matura-

tion, the cleavage-determining factors are either not yet definitely localized, or are capable of complete regulation, so as to assume in the fragment a disposition identical with that of an entire egg" (Wilson).

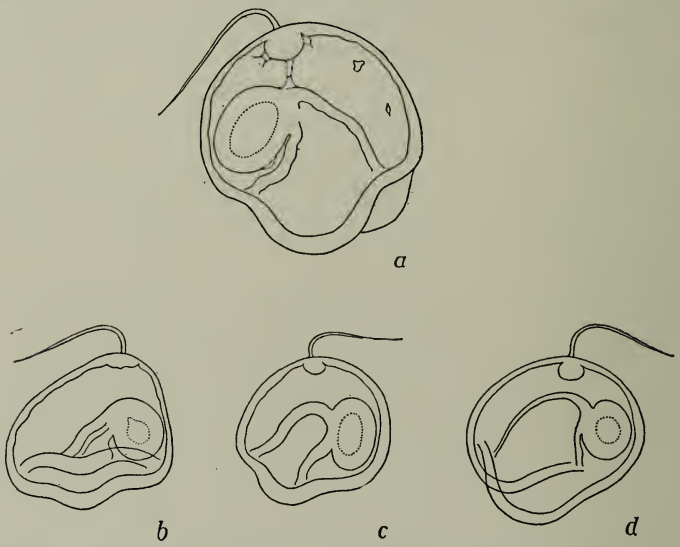


FIG. 86.—*a*, normal pilidium stage of Cerebratulus; *b*, *c*, and *d*, pilidia from egg-fragments. (After Wilson.)

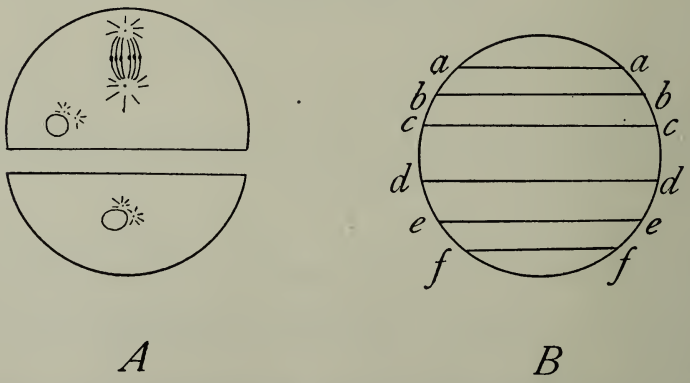


FIG. 87.—*A*, egg of Cerebratulus cut in two; first polar spindle present. *B*, same showing levels of cutting. (After Yatsu.)

Yatsu made a series of comparative experiments, cutting the unfertilized egg when the polar spindle was in metaphase at different levels (Fig. 87). Both fragments were then fertilized. The fragment above *a-a*, forms an embryo, but one without a gut

or an apical organ. This can safely be ascribed to the small size of the piece. The fragment above *b-b* produces a pilidium with a defective gut, but without apical organ. Here again the small size may be responsible for the defects, since in the light of the experiments with fragments below the equator that produce apical organs, it is improbable that the defects are due to "germinal localization" of apical forming materials. The fragment above *c-c* produces a pilidium with a gut only slightly deformed and an apical organ. Fragments above *d-e-f* produce normal pilidia.

The non-nucleated fragments, partners of the above pieces gave after fertilization the following results. The fragment below f-f, produced only a solid mass of cells. Its small size may account for this result. The fragment below *e-e*, produced an embryo without apical organ, but whether this latter defect is due to the absence of certain material is uncertain. The fragment below *d-d* may produce a perfect pilidium, but often the apical organ is not normal. Cuts above these levels give normal pilidia from the basal fragment.

Yatsu also found when large pieces were cut from the side of the egg, some before and others after the polar bodies had been formed, that the latter more often gave rise to defective embryos than did the former. This difference he is inclined to attribute to progressive localization effects. But he points out that this is by no means the only possible interpretation. The latter operation more often kills the eggs and the results may therefore be due to injuries different in kind from such as might result from localization conditions.

Yatsu ('04) has also made a detailed comparative study of the localization problem in the Cerebratulus egg by a series of operations on eggs at four different stages: (a) before the dissolution of the germinal vesicle; (b) at the metaphase of the first polar mitosis; (c) at the time of conjugation of the two pronuclei; (d) after the constriction of the first cleavage has appeared. The results were as follows:

(a) Since the germinal vesicle lies nearer the pole most of the cuts were in the antipolar hemisphere (the angle not determined). Thirty-five egg-fragments from the nucleated region developed up to the pilidium stage. Of these, 30 were normal, and 5 defective. (b) Sixty-five embryos developed from the

nucleated fragment, of which half (34) were normal, and half (31) defective. (c) Owing to the presence of the polar bodies the direction of the cut could be determined. As shown in Fig. 88*A* the operations were of three sorts. When cut in the plane *a-b*, four larvae were somewhat defective, one normal; when cut in the plane *c-d* (the polar region being removed) four larvae were normal, one showed some doubling; when cut in the plane *e-f*, seven larvae were defective, and when cut in the plane *g-h* eight larvae were defective, one normal. The most common defect in the last group (c) occurs in the ciliated lobes. The relation between this regional defect and the obliquity of the cut is suggestive, but not demonstrative. (d) Only three operations of this sort were made, but at another time ('08) ten further operations were carried out. All of them may be here taken together.

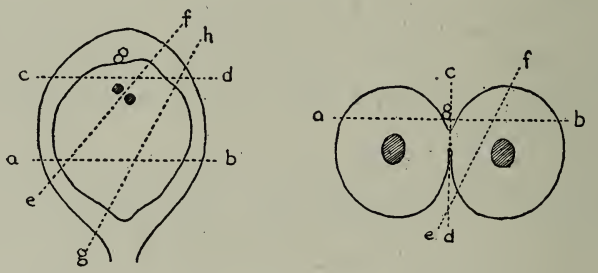


FIG. 88.—*A*, egg of Cerebratulus with two pronuclei present, the dotted lines show planes of cutting; *B*, egg in two-cell stage, the dotted lines show planes of cutting. (After Yatsu.)

One egg, cut in the plane *a-b* (Fig. 88*B*), gave a perfect pilidium, despite the fact that the polar region was removed. Two were cut apart in the plane of division (*c-d*). Each blastomere produced a pilidium with one lobe and no apical organ. One egg was cut in the plane *e-f*. The left fragment gave a dwarf, but perfect pilidium. In this case, material seemed to pass from the left into the right blastomere until they were of the same size. Presumably the separation had not been complete at the time of operation.

The foregoing experiments do not suffice to warrant detailed conclusions as to localization, but the effects of the operation are summarized by Yatsu as follows: (1) when the operation took place between the time of formation of the first and second polar

bodies the cleavage was regular (12 cases); (2) when the operation was made between the time of extrusion of the second polar body and the first cleavage, some eggs divided regularly, many irregularly. The relation of these differences to the resulting embryos is not stated.

The experiments show that changes are taking place in the egg at the time of polar body extrusion, and more especially after that event, of such a kind that fragments from the polar portion of the egg less frequently develop a normal digestive tract and lateral lappets than do the fragments from the antipolar region.

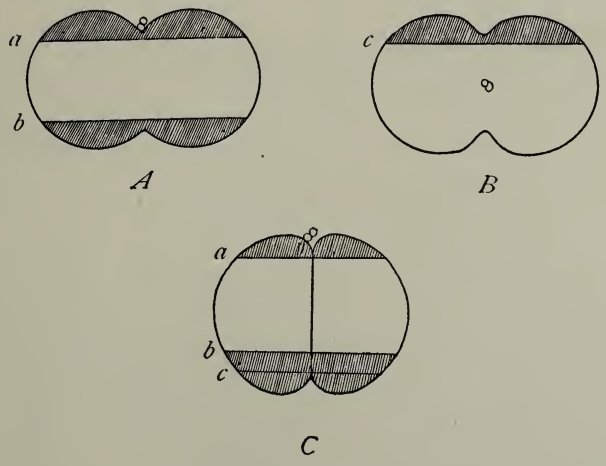


FIG. 89.—*A*, first cleavage stage of egg of Cerebratulus, the shaded areas indicate parts removed; *B*, same, seen from pole; *C*, two-cell stage—the shaded areas indicate parts removed. (After Yatsu.)

These indications are supported by further experiments by Yatsu and by Zeleny in which polar portions and antipolar portions were removed from the egg during cleavage. Such operations (Yatsu, '10), if performed as indicated by *a* and *b* in Fig. 89*A*, gave the following results:

The part above *a*, gave one perfect and two defective pilidia.

The part above *b*, gave 3 fairly normal pilidia, 1 pilidium with small gut, and 2 without gut.

As a control to these operations a portion was cut off at the side as shown in Fig. 89*B*. In two cases both embryos were normal.

The following operations were made when the division was completed (Fig. 89*C*), with the following results:

The part above the *a*-level gave 3 perfect pilidia.
The part above the *b*-level gave 3 pilidia with defective gut.
The part above the *c*-level gave 4 perfect pilidia.

The difference between *b* and *c* may be due to the part removed being so small in *c* that defects in the embryo were not noticeable.

Nevertheless, if the results were due only to a regional difference, the absence of the part below *c* might be expected to show a defect of the kind observed, since it is the most antipolar material present in the egg.

Zeleny's results on Cerebratulus marginatus of the Mediterranean agree in all essential points with the foregoing, except that he found a tendency to greater irregularity in the cleavage of the fragments removed at the time of, and immediately after, the polar body formation. After the second polar body is given off, up to the time of the first cleavage, Zeleny found that the fragments do not segment as wholes, but show very evident departures from the normal pattern; there was much difference in the extent of their departure, "from a possible whole cleavage through cases with a slight disturbance in size and position of cells, or rhythm of divisions up to a case with an open cup-shaped blastula of a purely partial type." However, no definite relation between the direction of this cut and the departures in the cleavage was evident. He also found that as soon as the egg begins to elongate for the first cleavage, and all through that stage, the fragments show unmistakably a partial cleavage indicating that changes had by this time taken place in the direction of the cleavage pattern. The change here is of the sort shown by the isolated blastomeres after the first division has been completed.

The Development of Fragments of the Egg of the Mollusc, Dentalium

When first set free the egg of Dentalium is somewhat irregular in outline, but soon rounds out, although it remains somewhat barrel-shaped (Fig. 90*a*). It has a broad, reddish-brown, pigment-band around the middle with a white polar (or apical) field and also a white antipolar (or basal) field. It had been

attached to the wall of the ovary at the more flattened base or antipole. The egg is surrounded by a jelly that swells in sea water. Sections of the preserved egg (Fig. 91*A*) show a very large nucleus, that lies near the center of the egg. The white area at the pole of the egg is due in part to a small disc of clear protoplasm that is continuous at its periphery with the ectoplasm of the egg, but the white area, as seen in the living egg, is larger than this disc that occupies its center. Around the nucleus, extending above it (apically) but not *below* it, is an

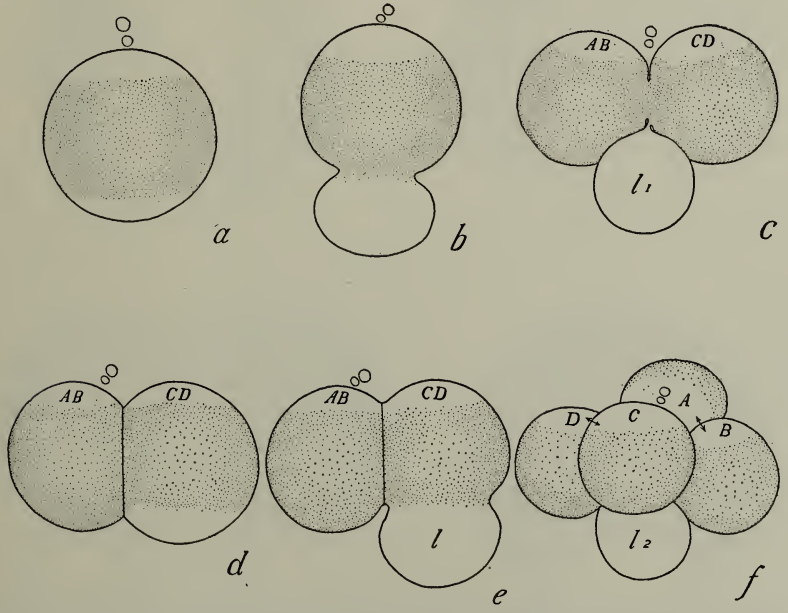


FIG. 90.—First and second cleavages of the egg of Dentalium showing the formation of the yolk-lobe. (After Wilson.)

abundant accumulation of yolk-spherules. Surrounding the basal pole is a mass of "dense almost homogeneous protoplasm of approximately the same extent as the white area seen in the living egg. This contains no yolk-spheres, and stains with great intensity with a strong plasma-stain like Congo red." The mass bulges slightly outward; it is sharply marked off from the yolk, and is continuous at its periphery with the thin ectoplasmic zone that surrounds the egg.

When the egg-nucleus breaks down a small polar spindle develops just below the polar disc (Fig. 91*B*). The spindle

remains in metaphase until the egg is fertilized. The first polar body is then formed, and is soon followed by the extrusion of the second polar body. The first cleavage, that begins about thirty minutes after the formation of the second polar body, starts at the pole and extends toward the basal field. At this

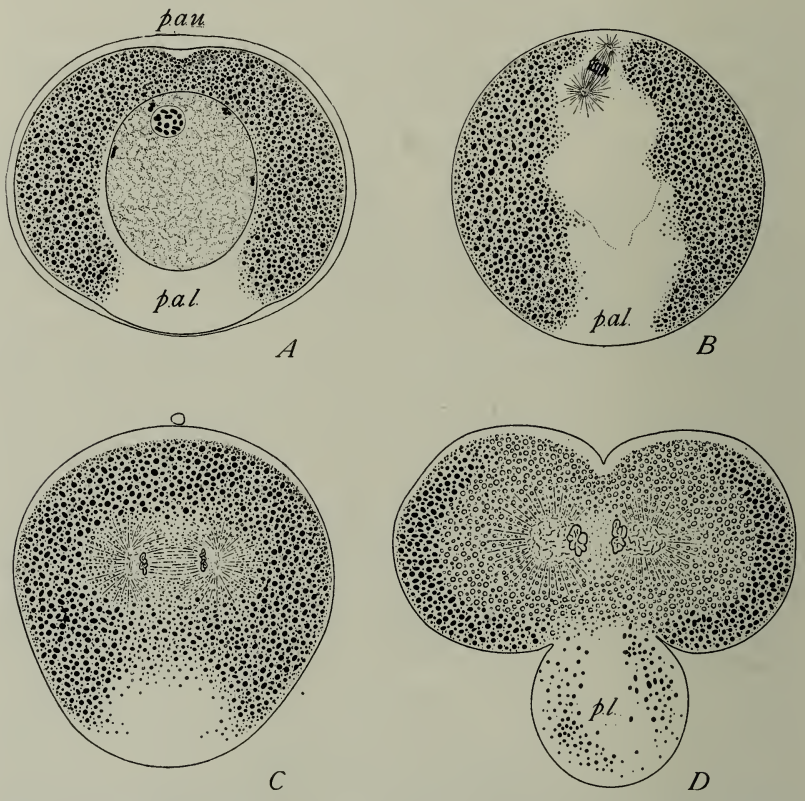


FIG. 91.—*A*, section through the mature egg of Dentalium with germinal vesicle; *B*, first maturation spindle; *C*, segmentation spindle preparatory to the first division; *D*, first cleavage with yolk-lobe. (After Wilson.)

time the basal field also begins to constrict by a circular, meridional groove to form a lobe including the white, antipolar material (Fig. 91*B* and *D*). This lobe, known as the yolk-lobe, contains, in reality, almost none of the yolk. Wilson calls it, therefore, the polar lobe, but because of its location, it is here referred to as the antipolar or basal lobe.

The lobe looks like a hernia in the "lower" hemisphere near

the antipole. There are a few facts connected with its appearance, and with its later relation to the median plane, to suggest that it starts in the region where a spermatozoön has entered the egg. That it is not simply due to a hole, made by the sperm in the ectosarc, is evident, because in other eggs with a yolk-lobe it may appear when the egg is incited to develop by parthenogenetic means. Nevertheless, it is possible that the surface changes connected with the development of the fertilization cone may fix the center of the future lobe and determine on which side of the antipole the yolk-lobe will develop. If this relation should be shown to hold, it would bring together a number of observations connected with the origin and fate of this curious body.

As the division of the egg proceeds (Fig. 90*b*) this lobe becomes more and more pinched off, until finally it remains attached by a thin neck to one of the first two blastomeres (Fig. 90*c*), the so-called trefoil stage. This process may be described in another way. As the cleavage furrow passes down it fails to cut into the basal material, and turns to one side leaving that material in only one of the two cells; but this description leaves out of account an essential part of the process; for, the lobe itself seems to constrict from the rest of the egg before it is reached by the cleavage furrow. Facts to be described later show clearly that while this constriction of the lobe is synchronous with the cleavage, it may take place independently of the cleavage furrow.

After the division is completed, the lobe becomes absorbed into the cell, CD, (Fig. 90*d*) to which it has remained attached. Two unequal cells, AB and CD result; one (CD) larger than the other (AB) by the amount of the material of the basal lobe. The second cleavage is ushered in by the reappearance of the lobe (Fig. 90*e*). The cells AB and CD divide into equal parts, except that the lobe remains attached to the D-cell (Fig. 90*f*), making that cell correspondingly larger, when, as immediately occurs, the lobe becomes absorbed into it. The D-cell marks the left posterior region of the embryo. The two lateral cells A and C, shift so as to lie at a slightly higher level, and come in contact to form the characteristic cross-furrow. The cells B and D come into contact on the antipolar side to form a cross-furrow (Fig. 92*A*) at right angles to the one above.

At the third cleavage the first quartet of micromeres (1*a*, 1*b*, 1*c*, 1*d*) is formed by a slightly unequal division, in a dexiotropic

direction, of the first four cells. The lobe appears again at this time (Fig. 92*B*, from below), but is smaller than before, and is less constricted off.

At the fourth cleavage (Fig. 92*D*) the basal cells A, B, and C, D, give off four members of the second quartet (*2a*, *2b*, *2c*, *2d*) by a somewhat unequal division of each. The *2d* cell is a larger cell than the other members of the quartet. It is labelled X in Fig. 92 and is the first somatoblast (Fig. 92*D*, *E*). When this cell, X, is produced, all of the white substance of the antipolar

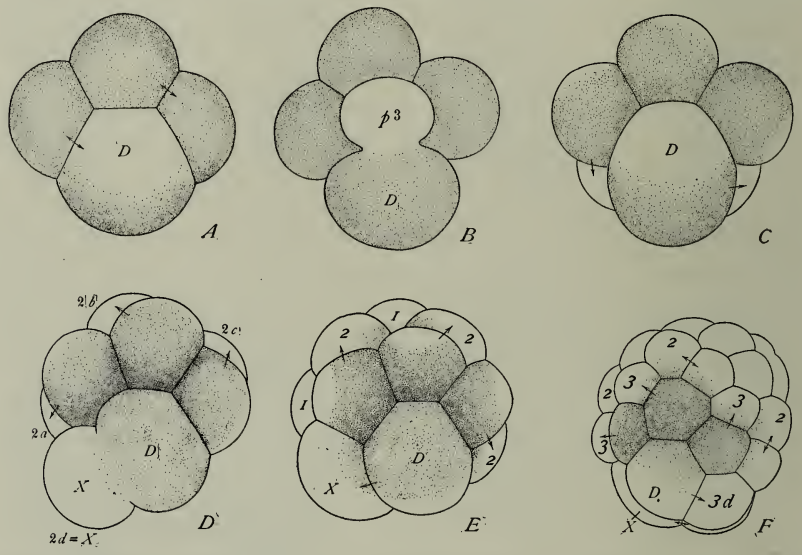


FIG 92.—*A*, four-cell stage of egg of Dentalium from lower pole; *B*, same, showing the second appearance of the yolk-lobe; *C*, eight-cell stage from lower pole, showing the absorption of the yolk-lobe into 4D; *D*, third division from lower pole, showing the formation of a large cell of the second quartette from D; *E*, same after the division; *F*, fourth division as seen from below; the third quartette of micromeres has formed. (After Wilson.)

field (or lobe) moves over to one side to be included in it. While this division is taking place in the basal cells, the cells of the first quartet divide leiotropically, to form the four primary trochoblasts that are slightly smaller than their sister cells more apically situated.

The fifth cleavage is dexiotropic (Fig. 92*F*, from below) in all the basal cells producing the third quartet (3a, 3b, 3c, 3d). Of these four cells 3d is the largest (Fig. 92*F*). At the end of the cleavage, the basal cells become superficially smaller by pushing more into the interior.

A fourth quartet is formed at the next cleavage giving rise to 4a, 4b, 4c, 4d. The last cell, 4d, corresponds to that cell in other molluscs and in annelids from which the mesoblast later arises.

In respect to the three color zones present in the ripe egg, Wilson says: "The upper zone (upper white area) is allotted to the first three quartets of ectomeres; the middle pigmented zone is mainly allotted to the four basal entomeres, though a portion also passes into the ectomeres of the second and third quartets; while the lower zone (lower white area) certainly passes mainly into the first somatoblast, 2d or X, probably in part into the second somatoblast, 4d or M, and possibly in part into the left posterior micromere 3d of the third quartet. This agrees in general with the history of the zones visible in the egg of Myzostoma as observed by Driesch." How far these regional differences are specific for the organs of the embryo into which they develop is shown by Wilson's experiments in which fragments of this egg were studied.

The embryo becomes ciliated about the tenth hour. At the end of 24 hours a well-developed trochophore is formed (Fig. 93*a*). The body has a blunt spindle shape and is encircled at the

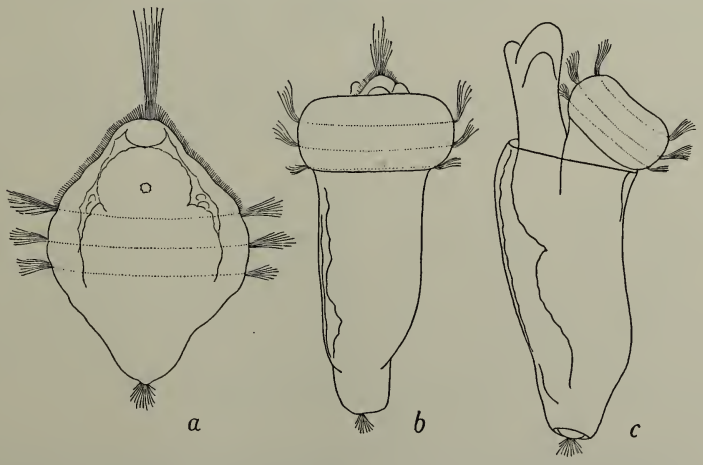


FIG. 93.—Three larvel stages of Dentalium, the last one, *c*, has undergone transformation. (After Wilson.)

equator by a broad prototroch, which carries three rows of large cilia encircling the body. Anteriorly the embryo is covered with

cilia, and at the apex there is a tuft of long sensory hairs. Posteriorly there are no cilia, but at the extremity there is a bunch of stiff sensory hairs. Later stages are shown in Fig. 93*b* and *c*.

Delage ('99) cut 18 unfertilized eggs of Dentalium into two pieces, and in six cases both fragments segmented. The first cleavage of the fragment was only "rarely" into two equal parts; more usually the fragment divided at once into four parts. It is possible that these fragments were polyspermic. Unfortunately, no information is given as to the details of the cleavage, or as to the type of cleavage of the nucleated and non-nucleated fragments. Normal as well as abnormal embryos were obtained from both fragments.

Information that is lacking in Delage's account is supplied by the later work of Wilson ('04), and the following statements and illustrations are taken from his paper. The eggs were cut in two with a scalpel, and the fragments were then fertilized. Both pieces often segmented. In Figs. 94, 95, 96, the position of the

FIG. 94.—*a*, cleavage of upper fragment, without yolk-lobe, of Dentalium egg; *a'*, *c*, cleavage of lower fragment with yolk-lobe; *b*, trochophore from upper fragment. (After Wilson.)

cut is indicated in the small circle placed near each cleaving fragment. The fragment that is drawn is indicated by a black dot. The pole is above. The egg-nucleus, after the extrusion of the polar bodies, lies in this region. The polar bodies are given off from the polar fragment after the operation. Their presence makes it possible to determine which of the two fragments contains the egg-nucleus, as well as its regional relations.

When the unfertilized egg is cut in two nearly equal parts in a horizontal plane, and then fertilized, the polar, nucleated fragment divides into equal parts as does the normal egg, except that no yolk-lobe appears (Fig. 94*a*). The second cleavage is again equal. The succeeding cleavages are spiral; quartets of micromeres being formed by alternating dexiotropic and leiotropic

divisions. Many of the embryos fail to develop, but others develop into actively swimming trochophores (Fig. 94*b*) that are never quite normal. The larvae sometimes agree precisely with those derived from whole eggs from which the yolk-lobe has been removed. These will be described later. In a few cases an apical organ is present. When the upper fragment is larger than the lower one, it may form a small yolk-lobe.

The lower (antipolar) fragment (Fig. 94a^1, *c*) may segment "in every detail like an entire egg of diminished size, forming the

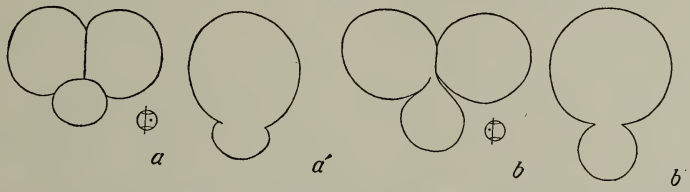


FIG. 95.—Cleavages of eggs of Dentalium cut in or near polar axis. (After Wilson.)

polar lobe in normal fashion, and may give rise to a dwarf larva nearly or quite normal in form and possessing an apical organ" (Wilson). Moreover, while there is much variability in the size of the polar lobe, it is often of exactly proportionate size, even when the section has passed above the limits of the white antipolar field. The form of the cleavage is, within limits, independent of the size of the piece; that is, both large and small fragments (¼ full size) may segment as wholes. Many of the embryos perish, and of those that live many are abnormal, but occasionally a dwarf embryo is produced that is a normal trochophore except in size.

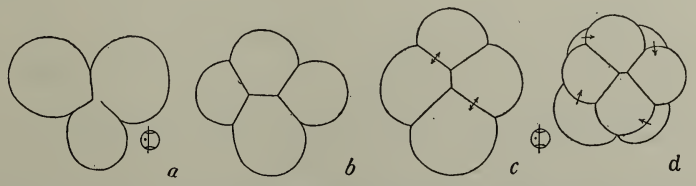


FIG. 96.—First, second, and third cleavage of fragment of Dentalium egg cut in or near polar axis. (After Wilson.)

When the egg is cut in two, parallel to the primary axis, i.e., "vertically," and the fragments are then fertilized, both halves may develop (one diploid, and the other haploid); both produce a yolk-lobe, if the antipolar white field had been cut in two. Cases of this kind are shown in Fig. 95*a*, a^1, *b*, b^1, and in Fig. 96*a*, *d*).

The yolk-lobe is sometimes relatively too small, but in other cases it may be of proportionate size. The later cleavage of both fragments from the same egg is like the normal cleavage in all respects (Fig. 96*b*, *c*, *d*). Some of the larvae from such a vertical section approach in form the normal trochophore.

The Development of Fragments of the Eggs of the Ctenophore, Beroë

It was shown by Driesch and Morgan ('95) that fragments of the unsegmented eggs of Beroë segment sometimes as wholes, and sometimes as parts of wholes. They surmised that this difference might depend on whether the cut was symmetrical with respect to the pole or lay on one side. The later experiments of Yatsu ('11, '12) have shown that this is true if the cut is made when the first cleavage is about to take place, but for oblique cuts the differences in the type of cleavage depend on the time at which the operation is performed.

The cleavage of fragments of the egg of Beroë was later studied more in detail by Yatsu ('11, '12), and also by Fischel ('98, '03) and by Ziegler ('98). Yatsu cut unfertilized eggs soon after being laid (the plane of the section was not determined) and in the two cases examined, the cleavage was normal. He also cut eggs in various planes after the two polar bodies had been extruded. Three eggs were cut vertically, i.e., parallel to the egg-axis. Normal cleavage followed, although the middle and end-cells on the cut side were somewhat smaller than their vis-à-vis, but the micromeres were the same in all (Fig. 97*a*). Six eggs were cut obliquely (Fig. 97*b*) with results as before. One egg was cut horizontally (Fig. 97*c*). The end-cells were small, but the micromeres were almost as large as in the normal egg.

At the beginning of the first cleavage three eggs, about to divide, were cut horizontally (Fig. 97*d*). The small polar fragment divided normally. Seven eggs were split in two in the plane of cleavage (Fig. 97*e*). Each fragment cleaved as a half. Further experiments of the same sort—more especially after the first cleavage had progressed some distance—were undertaken by Yatsu to see how far the subsequent divisions give proportionate cleavage patterns. Most of the sections were horizontal, and only the nucleated fragment segmented (Fig. 97*f*). Even when

the polar end was cut off after the cleavage-head had passed beyond the plane of section (Fig. 98*b*, *c*) the fragment gave a proportionate and whole cleavage. This is not at all inconsistent with the fact that if the dividing egg is cut in two in the plane of division each half cleaves as a half, for it is evident in the latter case that each half is more like the normal

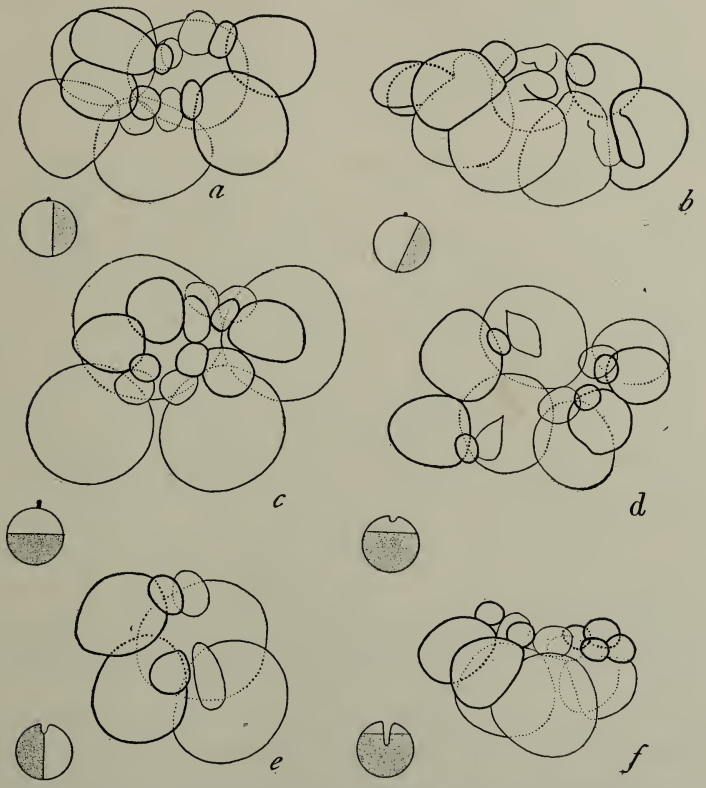


Fig. 97.—Cleavages of fragments of eggs of Beroë. The small figures to the left show the plane of cutting. (After Yatsu.)

½ blastomere; the mitotic figure is the same and the material is the same as that of the ½ blastomere; while in the former case the small polar fragment contains the two nuclei with their surrounding materials (although much reduced in volume), and these are symmetrically placed around the pole. If the size of the mitotic spindle is dependent on the cell-contents, and if the cleavage is determined by the spindle rather than by the material

of the cytoplasm, one can understand to some extent at least why the cleavage of these polar fragments is proportionate.

There is another important fact shown by these experiments of Yatsu. If the section is made when the first cleavage is just beginning, the nucleated (polar) end contains a great part of the ectoplasm which at this time has moved into the polar region, yet the micromeres are no larger than in the case when the section is made towards the end of the first cleavage, at which time much of the ectoplasm has left the polar region and moved into the antipolar field. Evidently, the amount of ectoplasm is not in

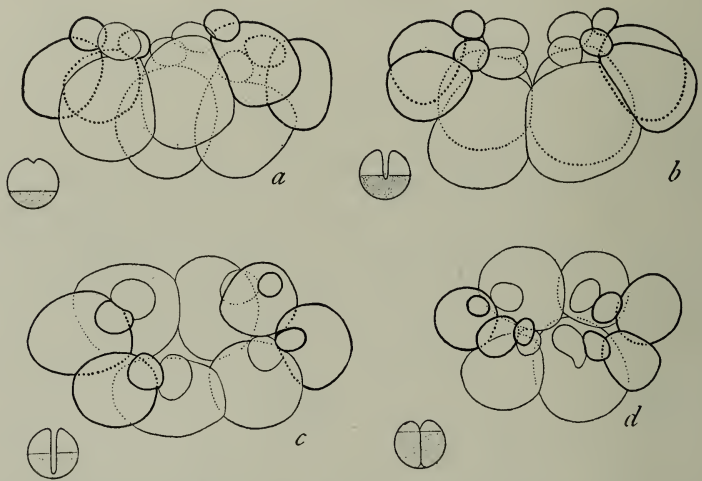


Fig. 98.—Cleavages of fragments of eggs of Beroë, cut at various stages during the first cleavage. The small figures to the left show the plane of cutting. (After Yatsu.)

itself the factor that determines the size of the micromeres, although in the normal egg they appear to be largely composed of this material.

The Development of Fragments of the Eggs of Sea-Urchins

The experiments with fragments of the sea-urchin's eggs were the first of the kind to draw general attention to the importance of this method of studying the changes in the egg, prior to its cleavage. The work of Boveri ('89, '96, '14, '18), Morgan ('93, '95), Driesch ('96), Delage ('99) on the sea-urchin's egg showed that the nucleated fragments of this egg may develop into whole

embryos of small size. It was also found that even the non-nucleated fragments might also divide if entered by a spermatozoön, and it was supposed that they also might produce small embryos.

If the eggs of the sea-urchin are shaken violently in a small tube before fertilization they may be broken up into fragments of various sizes; some of the fragments contain the egg-nucleus, others are without a nucleus. It is not known explicitly how each of the two kinds of fragments behaves, but it is known that the fragments that contain both the egg-nucleus and the sperm do develop, while those that contain only the sperm may segment, and the larger ones, at least, are supposed to gastrulate, and even to produce plutei.

The cleavage of egg-fragments of sea-urchin's eggs, obtained before fertilization, has been studied by Morgan ('93), Driesch ('96) and Delage ('99). Morgan described the cleavage as very irregular. The pattern was not like that of the whole egg. Driesch examined eggs of two sorts. One lot of eggs was shaken an hour after fertilization, hence just before the first cleavage is expected. Seven types of cleavage patterns were found, all "partial" or irregular. Some of the larger pieces especially showed at times a close approach to half cleavage. Driesch ascribes the differences in cleavage types as due to the various regions of the egg from which the fragments have come, and since the eggs were fragmented just before the first cleavage was due, this seemed a probable explanation in the light of evidence from other sources. Driesch also studied the cleavage of eggs that were broken into fragments before fertilization. He found that these fragments also showed great variability in their cleavage pattern. The same types that he had obtained from fragmentation of later stages (described above) reappeared here also. In a few cases, especially in the larger fragments, normal cleavage was observed. He concluded, nevertheless, that partial and not whole cleavage is the rule and inferred that the cleavage pattern is predetermined in the egg.

Delage ('99) cut each egg into two parts—a method that is better suited to give an answer to the question involved. The fragments were then fertilized. In six cases both fragments divided. Delage states that "the cleavage takes place as in the normal egg," but no details are given and, judging from the

result he records for fragments of other kinds of eggs, it seems probable that Delage did not follow the cleavage in sufficient detail to answer the question as to whether the characteristic features of the cleavage were those of the whole egg.

From the foregoing evidence it is uncertain, I think, whether fragments of the egg of the sea-urchin that are obtained after the polar bodies have been extruded segment as parts or as wholes. It is quite possible that the rough treatment employed, when the eggs are broken by shaking, injures them and disturbs their materials to such an extent that the irregularities observed in the cleavage are due to such disturbances rather than that they represent "partial cleavage." The method of cutting the egg is undoubtedly better, and has given more definite information. These observations may now be given.

Chambers and Ohshima ('22) removed the nucleus of the sea-urchin egg with a minimum amount of cytoplasm. In some cases the non-nucleated part contained as much as $\frac{4}{5}$ of the entire egg. Such a piece developed after fertilization into a larva about half the normal size. The nuclei in the cells were "abnormally small." Other eggs were deprived of about half of their cytoplasm. The larvae were about half as large as the normal larvae, but the nuclei were equal in size to those of the normal control. Without further information as to the number of cells present in these "larvae" and as to the actual size of the nuclei, it is not possible to draw any inference from the evidence.

The development of non-nucleated fragments of another sea-urchin, Lytechinus (Toxopneustes variegatus), has been examined by Taylor and Tennent ('24). In this species the polar bodies are retained in place and furnish a means of locating the plane of the cut. The cutting was done with a micro-dissection machine, which permits an accurate determination of the plane of operating. When the operation is done slowly, the surface layers are brought together before the final separation takes place, and when inseminated, a membrane forms over the whole fragment which has rounded up. Presumably the old surface layer covers the side of operation. On the other hand, if the cutting is done quickly with a very sharp needle, the fragment remains flattened on one side and no fertilization membrane forms on that side.

The nucleus in this egg, as in other sea-urchin eggs, may lie at first in any position with respect to the primary axis. Some

of the eggs were cut in a vertical plane (parallel to the egg axis); others at right angles to this (horizontal). The nucleus, owing to the excentric and irregular position might lie in either fragment. After insemination, either fragment might develop, both in other cases. Gastrulae and plutei (72 hours) were obtained from both kinds of fragments. One of these must be diploid, the other haploid (monospermic). Except for size, the plutei could not be distinguished from normal ones.

In respect to the cleavage it was found that in every case, when both fragments of the same egg divided, the pattern of one was like that of the normal egg, while the other fragment did not produce micromeres at the fourth division, but the antipolar cells divided equally, and shortly after the division of the four cells of the other hemispheres. This statement is superseded by later work to be discussed below. Furthermore, the original polarity of the egg does not necessarily persist in the fragment. With but one exception the first two planes of cleavage were at right angles to the plane of section, and the micromeres, when formed, appeared at an intersection of these two planes. Consistent with this is the fact that, with one exception, the long axis of the first cleavage spindle was in the major axis of the fragment. Since, as stated above, most of the fragments developed normally, it would appear to follow that a new axis is formed in the fragment which becomes the axis of the embryo; hence either there is no superficial area, if such exists in the unfertilized egg, that is essential to organ formation, or it is capable of adjusting itself to the new conditions imposed on the egg. On the other hand, there might be materials within the egg that are sorted out just before or during the cleavage stages, and if so their distribution might be determined by the presence of the cut surface, or by the shape or compression of the egg, or by the sequence of the cleavages. These possibilities will be considered after other observations have been given.

The nucleus of the Arbacia egg also lies excentrically, but whether more often in the polar hemisphere than in the antipolar is not known. Harnly ('26) cut the eggs free-hand under a binocular microscope. The first cuts were made approximately through the middle of the egg (Fig. 99*a*) and then the fragments were fertilized. In 30 cases the nucleated piece gave a typical cleavage.[1] The first cleavage was at right angles to the plane of

[1] Fifty eggs formed endoplasmic buds, and thirty-eight divided irregularly.

cutting; the second at right angles to the first and to the plane of cutting; the third cleavage was equatorial. At the fourth cleavage four micromeres formed at one side, four macromeres next to them, while the four cells in the other hemisphere divided equally.

In another experiment the two halves of each egg were followed and the cleavage of both recorded. One fragment gave a typical fourth cleavage; the other half produced two tiers of eight equal cells—i.e., no micromeres were present. In still another experiment the nucleated and the non-nucleated fragments of the same egg were kept together; the former, 23 in number, gave a typical cleavage pattern, the latter, 28 in number, gave two tiers of eight cells.

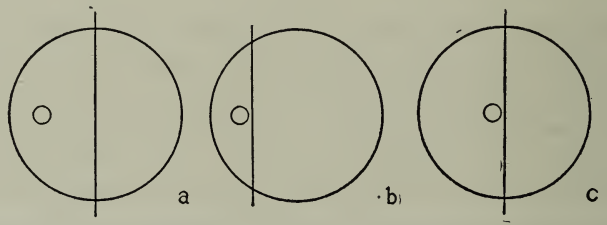


Fig. 99.—Eggs of the sea-urchin, Arbacia, showing the planes of cutting. (After Harnly.)

A possible explanation of these results was suggested. If there is some material in the interior of the egg that is responsible for the micromeres, it will come to be contained, as a rule, in the nucleated half, provided it too comes to lie somewhat excentrically and on that side where the nucleus lies. The nucleated half will then be expected more frequently to contain this material than the non-nucleated half.

This interpretation can be tested by cutting an egg with very excentric nucleus near the nucleus. The non-nucleated piece will then be expected, sometimes, to contain the postulated material. In fact, the material might at times be divided, and both pieces receive some of it. Cuts were made as shown in Fig. 99*b*. The non-nucleated piece contained about two-thirds of the material: this monospermic fragment then gave a typical cleavage, i.e., one with micromere formation.

Other experiments were made in which the eggs were divided into equal parts, but the plane of cutting passed near to the

nucleus, parallel to a line joining the nucleus and the center of the egg (Fig. 99*c*). Here the position of the cut, relative to the region between the excentric nucleus and the egg center, is somewhat uncertain. Both halves cleaved as wholes in 20 cases; in two cases the nucleated piece cleaved as a whole, but the other piece did not divide. In six cases the non-nucleated pieces divided as a whole, etc. In other cases, only two micromeres developed. Omitting these rarer cases, the results can be brought into line with the hypothesis of an interior material; for, it is clear that the cuts were such that the material if present might sometimes be divided and each fragment receive a part of it.

Other operations were made on eggs at intervals *after* fertilization. As is known, the egg-nucleus moves, or is carried into the axis of the egg a few minutes after fertilization, and lies excentrically in the polar hemisphere. Its constant position permits localizing the plane of cutting, but in the method of free-hand cutting there is a chance that the egg turns so that occasionally the nucleus may come to be in the presumptive antipolar fragment. Owing to the presence of the fertilization membrane, the two parts remain in contact, compressed against each other as much as are two blastomeres. Nevertheless, as the results show, this compression does not affect the cleavage-pattern, except as to details. A half that is hemispherical in shape may cleave as does a whole, spherical egg (Fig. 100*b*).

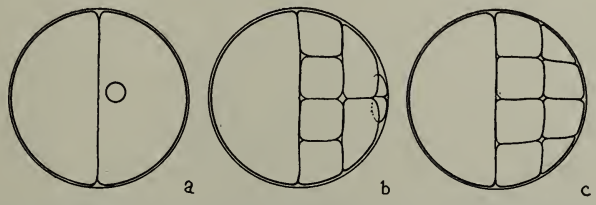


Fig. 100.—Segmentation of the egg-fragments of Arbacia in contact with unsegmented pieces, both within the same membrane. (After Harnly.)

When *fertilized* eggs were cut into two equal parts through the equator, the first plane of cleavage was always perpendicular to the cut surface. The second cleavage was at right angles to the first and to the cutting plane. The third was equatorial. At the next division there were found, in nineteen cases, 16 equal cells (Fig. 100*c*). This is expected if the cut had passed between the segmentation nucleus and the postulated micromere material.

But in five others there were four micromeres at the outer surface of the egg, i.e., opposite the cut surface. These are unconformable cases, unless, as suggested above, the cut was not completed as intended, and the nucleus and micromere material were both included in the antipolar fragment.

In a later series of experiments (unpublished data) the fertilized egg was cut at the equator at the time when the amphiaster is present, which lies more in the polar hemisphere. Thirty-seven nucleated fragments divided into 16 equal cells, i.e., without micromeres. Twenty-three of these gave rise to swimming blastulae, twenty-two of which did not gastrulate. One egg, cut slightly lower, gastrulated. Three eggs, cut slightly below the equator and nearer the antipole, gave micromeres at the cut surface, and all gastrulated.

These results show at least, that when the amphiaster is present, some sort of material, connected with the formation of the archenteron, lies below the center of the egg, and is brought to the surface (or determines that the spindles are brought to the surface) at the 16-cell division. It may be postulated that this material has developed in this position after fertilization has taken place, but it is also evident, that the presence of such material at this time is consistent with Harnly's interpretation of its presence at a still earlier stage in a corresponding position with respect to the nucleus.

One further point may be added. Occasionally the two halves, apparently after complete separation, flow together. The eggs may then give normal cleavage. Therefore, whatever determines the type of cleavage, it is of such a sort that if divided into two parts, it may be reunited without affecting the end result.

The results by Taylor and Whitaker ('26) on the eggs of the starfish, Patiria miniata, and two species of sea-urchins furnish further data bearing on the interpretation of polarity in these eggs. The cutting was done with a micromanipulator. The first and second planes of cleavage pass at right angles to the plane of section, irrespective of the position of that plane in relation to the primary egg-axis. This happens even in fragments that appear to be completely spherical before the cleavage. It is further reported, both for the starfish and sea-urchin, that gastrulation takes place on the cut surface. Unless by chance these cases (24 in number) happened to be those in which the

cut-surface is the antipolar end of the fragment, it follows, that, after an operation of this kind, a new gastrula axis may be established in relation to the cut-surface. It might be possible to explain these cases in relation to the presence of micromere material in the interior of the egg, but only on the supposition that the position taken by this material is affected by the cut surface or by the formation of the first two cleavages, so that it becomes oriented towards the cut surface before the time of formation of the micromeres, or, in their absence, of the gastrula material.

In a recent paper by Taylor, Tennent and Whitaker, in which a large number of carefully planned operations were made on the eggs of Lytechinus, the conclusion is reached that "in this egg there is no localization of micromere-forming material. There is no evidence that micromere-forming substance has been differentiated before fertilization." They believe that there is an "epigenetic" development of micromere material. Its development must then be supposed in some unknown way to be determined either by the cut surface, or to have some indirect connection with the presence of this surface.

An examination of the cleavage of the normal eggs of Lytechinus showed that the four micromeres do not always appear. There may be one, two or three of them. Since these eggs developed into normal plutei, it is evident that the presence of micromeres as such, is not essential, but it does not prove that some materials of this sort, if such exist, may not have been already present in the micromere region of the egg. It was also found that there was much variation in the fragments with respect to the number of micromeres that appeared. Nevertheless, since the results show that micromeres may develop on fragments from any part of the egg of Lytechinus, there can be no prelocalization of such material before cutting.

Fertilization of Non-nucleated Fragments of the Egg of One Species of Sea-Urchin by Sperm of Another Species

Boveri ('89, '96, '14, '18) carried out the ingenious experiment of fertilizing the non-nucleated fragments of an egg of one species of sea-urchin by sperm of another species in order to discover whether the characters of the larva are determined by the protoplasm, or by the nucleus, or by both.

It is true, that this question is to-day definitely settled by Mendelian crosses where single differences are involved, and unless there is something unknown and peculiar where species are concerned there is every expectation that, given sufficient time, the nucleus will determine the character of the structures produced by the cytoplasm; but at the time when Boveri first made his experiment all this was not known, or at least not appreciated. Boveri himself held later ('06) that "fundamental characteristics" of the organism are determined by the cytoplasm, while the smaller details in which species differ from each other are regulated by chromosomal activities. But this point is scarcely involved in the experiment in question, since the "fundamental" structures are the same in both species of sea-urchin used for the experiments. The real question to-day is only whether the influences under which the cytoplasm of the egg has been produced will affect the character of a pluteus that contains a nucleus of another species.

Boveri's experiments were made with the eggs of Sphaerechinus fertilized by the sperm of Echinus. The normal pluteus of Echinus is shown in Fig. 101*a* and *d* in side and in front view; that of Sphaerechinus in Fig. 101*c* and *f*; and that of the hybrid in Fig. 101*b* and *e*. A hybrid pluteus of "pure" Echinus type from an egg-fragment of Sphaerechinus fertilized by a spermatozoön of Echinus is shown in Fig. 102*a* and *b*, and another similar hybrid is shown in Fig. 102*c*. Most of the small plutei that developed from cross-fertilized fragments were intermediate in form and in the structure of their skeleton, but a few were found, like those in Fig. 102, that were purely paternal, and Boveri supposed that the latter were derived from the non-nucleated egg fragments of Sphaerechinus that had been fertilized by the sperm of Echinus. He concluded from this evidence that the nucleus determines the character of the pluteus, even when the cytoplasm belongs to another species, in so far as the two species in question differ from each other.

The validity of Boveri's evidence was questioned by Seeliger ('95, '96) and by Morgan ('95) on the grounds that the full-sized hybrid plutei, derived from this cross, sometimes produce larvae whose skeletons are purely paternal. Boveri's general conclusion was not challenged, but the evidence for it was questioned. In fact, while it now seems highly probable that paternal

larvae might be produced from non-nucleated pieces, depending in the main, on how far the earlier influence on the cytoplasm carried over to the pluteus stage, yet the evidence to prove this was

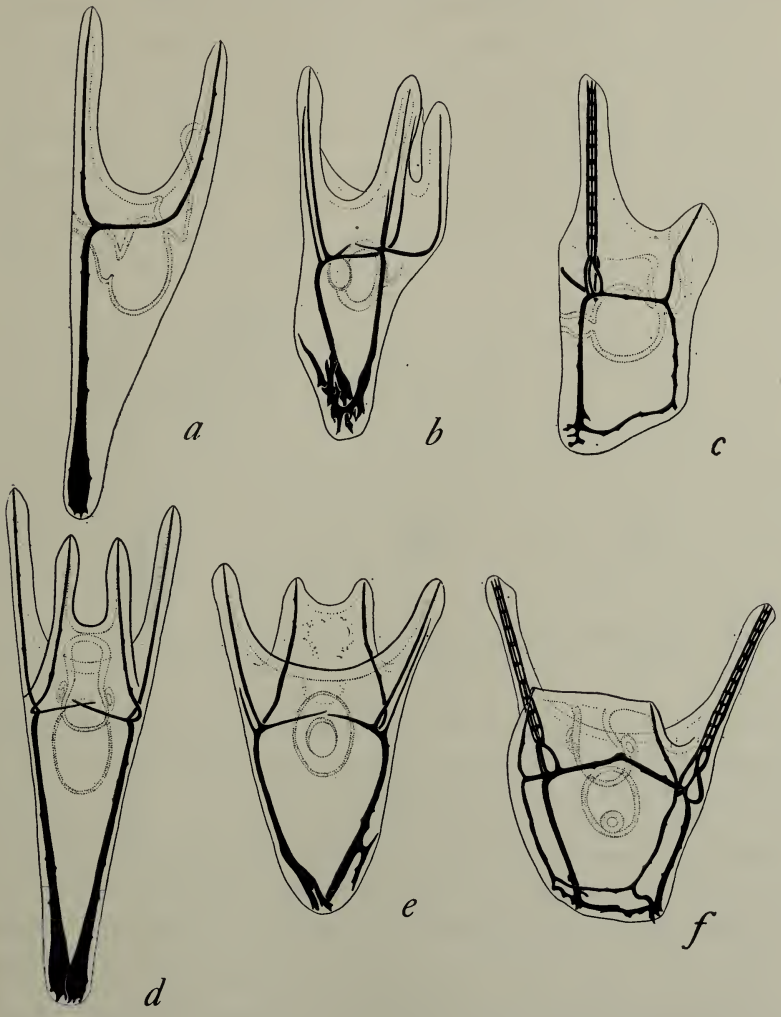


FIG. 101.—Pluteus of Echinus, *a*, in side view and, *d*, in oral view. Pluteus of Sphaerechinus, *c*, in side view and in oral view, *f*. Hybrid pluteus between Echinus and Sphaerechinus, in side view, *b*, and in oral view, *e*. (After Boveri.)

inadequate under the conditions of the experiment. Boveri was loath, at first, to recognize the value of the evidence advanced by Seeliger and Morgan, but in his last paper he is more inclined to

give credence to it. In fact, he later concluded that the hybrid plutei with paternal skeleton had indeed come from nucleated fragments.

Boveri continued for several years to search for convincing evidence to settle this question. He tried especially the method of isolating fragments of eggs in which no nucleus could be seen. This work is extremely laborious and disheartening, since even when embryos are obtained few of them reach the pluteus stage. It was not until the winter of 1911–12 and again in 1914 that he finally obtained the evidence he sought. The new results were published in 1918 a year after his death. He had found that

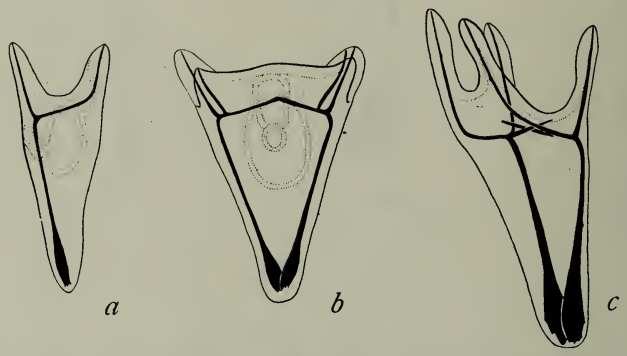


FIG. 102.—Plutei from fragments of the eggs of Sphaerechinus, fertilized by the sperm of Echinus. (After Boveri.)

none of the hybrid larvae from non-nucleated fragments develop beyond the late blastula or early gastrula stage; hence they do not furnish the evidence required.

In the course of this work Boveri went so thoroughly into all sides of the question involved that his investigation stands as a model of the highest type of embryological inquiry. It showed throughout a freedom from bias, a critical thoroughness of procedure and a resourcefulness of methods that call for the highest admiration. The clue to the final solution Boveri found in a more detailed study of the fragments of the Sphaerechinus eggs that were *apparently* without nuclei. Most of these fragments produced sickly blastulae that went no further, or, at most, formed the beginning of the gastrula stage, but in addition there were a few well-formed plutei. These were not all of paternal form, but on the contrary most of them were clearly intermediate in

their skeletal form as in the case when an entire egg of Sphaerechinus is fertilized by a sperm of Echinus. These plutei were killed and stained, and it was found that their nuclei were as large as those of a hybrid from a nucleated fragment of the same size. In other words, they were diploid. It seemed probable, therefore, that the supposedly non-nucleated pieces had really contained the normal number of chromosomes. Delage, in fact, had noted earlier that an apparently non-nucleated fragment might contain the constituents of the nucleus if the nuclear wall had broken down as a result of the shaking. Boveri also found later that when the eggs of Echinocardium are shaken the nucleus disappears from view in many eggs, but when killed and stained a group of chromosomes could be detected, as Morgan had earlier shown for starfish eggs. This mass resolves itself into its constituent chromosomes when the egg is fertilized, and combining with the sperm-nucleus forms a normal segmentation nucleus. Baltzer ('10) had in the meanwhile repeated the experiments of isolating supposedly non-nucleated fragments, crossing them with sperm of another species, and had found that all but two died as blastulae. These two, killed as plutei and stained, were found to contan full-sized (diploid) nuclei, and must, therefore, have contained originally chromosomes derived from the eggs.

Boveri ('18) repeated his isolation experiments once more. Two hundred egg fragments apparently without nuclei were isolated. From them eleven well-developed plutei were obtained. Eight of them had large nuclei, and the fragments from which they came must have had invisible, maternal chromosomes when isolated. Of the three remaining plutei two had both larger and smaller nuclei. They were, therefore, of doubtful origin, and may have come from partially fertilized egg-fragments, or from eggs whose nuclei had been in the form of isolated vesicles, as rarely occurs. The third pluteus having small nuclei showed probable indications of hybrid origin in its skeleton and is, therefore, also of doubtful value as evidence.

Finally, Boveri returned once more to mass-cultures of egg-fragments. Eggs of Sphaerechinus were broken up and fertilized in some cases with Echinus sperm, in other cases with Strongylocentrotus sperm. At intervals large numbers of fragments were preserved. Judged by the standard of nuclear size, he found that both the fertilized non-nucleated and nucleated fragments develop

at first at the same rate. But when the blastula stage is finished, the small-nucleated fragments cease to develop, while the others go ahead to form plutei. The oldest of the small-nucleated embryos stopped developing during the gastrula stage—only two showed traces of the triradiate spicules. This evidence, added to the rest, shows that the non-nucleated fragments fertilized by the sperm of the other species are unable to reach the pluteus stage. It seems probable that the foreign nucleus alone is unable to impress its characteristics on the cytoplasm that has developed under the influence of its own nucleus. But why it fails to do so is not at all evident in the light of the characters of the hybrid pluteus from whole eggs that are intermediate. Here the paternal chromosomes even in the presence of the maternal chromosomes are able to affect the character of the hybrid pluteus.

One further source of evidence must be added. Non-nucleated fragments of Sphaerechinus, fertilized by their own sperm, are able to produce plutei that have, of course, the characteristics of the species. Furthermore, Boveri found ('14) that the non-nucleated fragments of Sphaerechinus eggs fertilized by the sperm of a closely similar species, Strongylocentrotus, also produced plutei. The plutei of these two species are so similar that it is not possible to determine whether the monospermic hybrid plutei are more paternal than the ordinary diploid hybrid pluteus. Nevertheless, the result shows that the nucleus of one species and the cytoplasm of another species may combine to form a pluteus. Why, then, does not the more extreme combination develop as far? We can only conjecture that it is because of some secondary failure of this combination to reach this stage. It does not seem to be due to any very "fundamental" conflict between the nucleus and the cytoplasm, because a pluteus does develop in the other combinations.

In the light of his own results Boveri considers adversely the evidence that Godlewski has furnished relating to the fertilization of supposedly non-nucleated fragments of the eggs of the sea-urchin, Echinus, by the sperm of the crinoid, Antedon. Godlewski ('06) found that, while most of such fragments died after fertilization in early stages, four reached the gastrula stage, and that these were of purely maternal type (Fig. 103)—that is, the foreign sperm had failed to impress on the protoplasm any of the characteristics of its species.

Since Godlewski made no measurements of the nuclear size, Boveri believes that there can be no doubt but that these supposedly haploid gastrulae came from fragments whose chromosomes were present even though invisible in the living fragment. The probability of the correctness of Boveri's diagnosis is very great in the light of the other evidence furnished by Godlewski

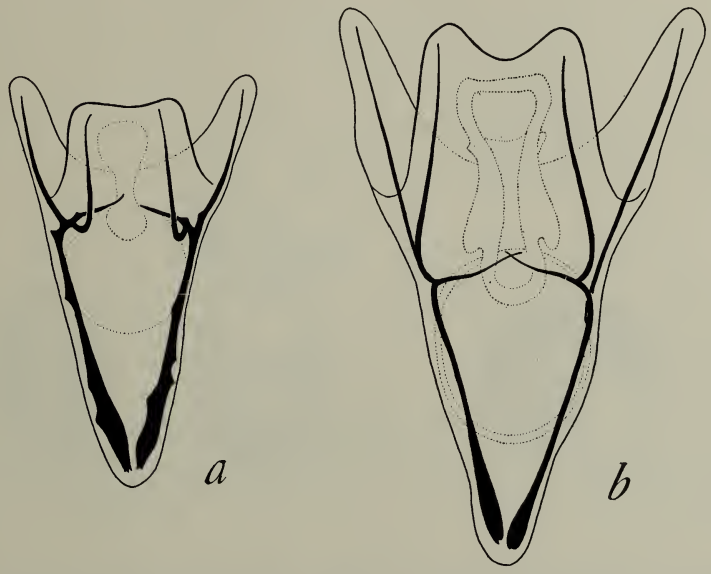


Fig. 103.—Two plutei from eggs of Echinus, fertilized by the sperm of Antedon. (After Godlewski.)

and by Baltzer, namely, that the hybrids from whole eggs (Fig. 103*b*) are also strictly maternal.[2]

The Development of a Part of the Egg of the Salamander, Triton

Spemann ('14) obtained an embryo that developed from a part of a Triton's egg that contained one sperm, and was without an egg nucleus. His method was as follows: A hair was tied around the egg immediately after fertilization and tightened until

[2] Tennent ('24) has reported some preliminary crosses between the non-nucleated fragments of Lytechinus eggs and Tripneustes sperm. Fry has reported similar experiments between fragments of Echinarachnius eggs and Arbacia sperm. Later stages were not obtained.

the egg was constricted into two parts of unequal size. More than one sperm enters the egg of Triton as a rule. The point of penetration is often marked by a dark fleck on the surface (Fig. 104*A*). The location of the polar spindle of the egg is indicated by a light area at the pole. One portion contained the light polar field, and presumably the egg nucleus, and one or more spermatozoa; the other portion contained a single sperm as indicated by its point of penetration. Both portions began to divide (Fig. 104*B*). Each produced an embryo (Fig. 105*A*). In the majority of cases one or the other half dies, or does not segment, and only very rarely does the monospermic portion develop as far as the embryonic stage.

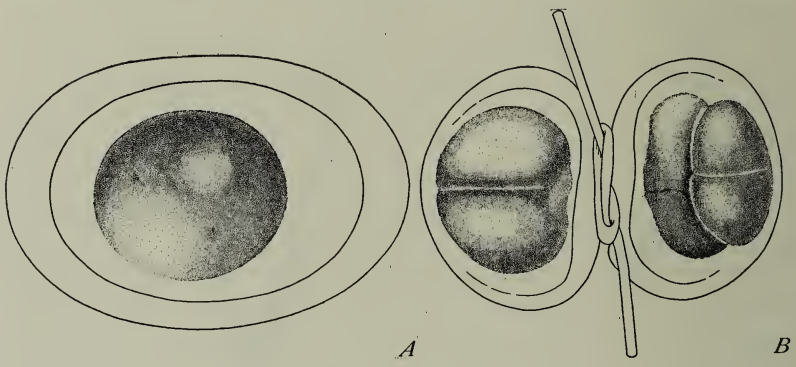


FIG. 104.—*A*, egg of Triton, showing a lighter spot where the polar bodies have been given off, and two dark spots where two spermatozoa have entered. *B*, later leavage of an egg that has been constricted into two parts after fertilization; one cagment is monospermic, the other contains the egg-nucleus, and has also been fertilized. (After Spemann.)

Baltzer ('22) has repeated this experiment and obtained in one instance a dwarf larva that developed as far as metamorphosis, when it died. It was less active than a normal larva, and moved more slowly. An examination of its nuclei showed that they had only half the volume of those of a normal Triton.

Spemann has also made some observations on the retarded and secondary nucleation of the "non-nucleated" portion of an amphibian egg that was separated into two parts only partially by a constriction from the nucleated part. If, immediately after fertilization, a loop is tied around the egg, and then is slowly tightened, the egg may be constricted so that it assumes a dumb-bell shape (Fig. 16*A*). The part containing the egg-nucleus

that had been fertilized by one of the sperm-nuclei divided into a small and a large blastomere. At the second cleavage, the small blastomere divided into equal parts, the large one into a small and a large blastomere. The divisions may continue until a later division cuts apart the dividing from the not segmented half by a cell wall passing across the connecting stalk. This is

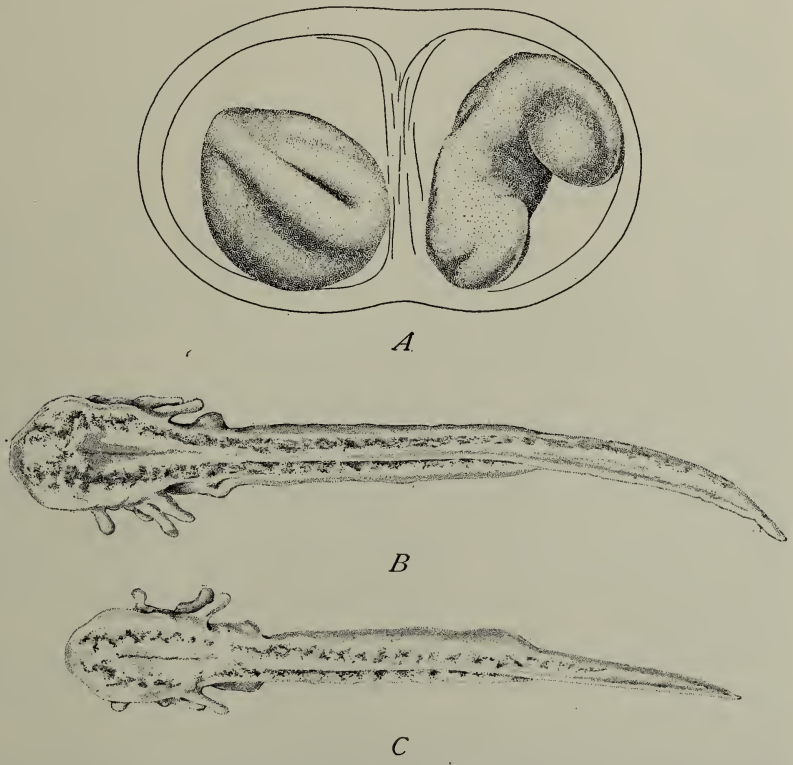


FIG. 105.—*A*, two embryos from fragments of the same egg, one of which is haploid (monospermic) and the other diploid. *B*, later stage of diploid larva; *C*, later stage of haploid larva. (After Spemann.)

accomplished because one of the daughter nuclei involved in this division has passed into the non-nucleated portion. The non-nucleated half now begins slowly to segment. What it produces, depends apparently on what portion of the fertilized egg it represents. If the constriction lies in the plane that corresponds to the future median plane of the body, twin, diploid embryos would be expected, if they arose as described above. If the con-

striction is across the median plane of the future embryo then only one embryo develops which may be either one of the two original halves. The result shows that whole development depends on the egg-plasm, and not on the kind of nucleus that reaches particular regions.

There is a further point of interest in this result. The nucleus that passes over the bridge may be one of the nuclei of the four, or eight, or sixteen blastomeres, yet any one of them seems capable of giving rise to a complete embryo. This furnishes further evidence that the nuclei have not undergone progressive changes during segmentation, as Weismann's mosaic theory postulated.

It had been found by Gunther Hertwig ('13) that, after treatment with the rays of mesothorium, unfertilized frog's eggs may begin to develop, but the embryos die in the early stages, living at most for only three or four days. But if the eggs are treated for a longer time with a stronger sample of mesothorium, the egg-nucleus is so injured that it takes no part in the development, or else is killed. Nevertheless, if these eggs are fertilized, a sperm-nucleus takes the place of the normal segmentation nucleus, and a haploid embryo develops. These embryos did not live longer than ten days. Paula Hertwig ('22, '23) states that of the several thousand of haploid larvae of the frog that Oscar Hertwig ('11), Gunther Hertwig ('13) and those she herself has obtained, none have gone further than the younger stages of development.

Paula Hertwig ('23) made a renewed attempt to obtain haploid embryos and larvae from denucleated eggs of one species fertilized by sperm of another species. Since normal hybridization is not very successful between these species it may seem doubtful whether haploid hybrids were to be expected. On the other hand, it is conceivable that in diploid hybrids the presence of the maternal chromosomes might conflict with the paternal, while in the denucleated eggs, the former influence being absent, there might be a better chance for the paternal chromosomes to change the cytoplasm "after its kind."

When the denucleated eggs of Rana arvalis were inseminated with sperm of Rana temporaria, many eggs developed, but the embryos died after eight days. The normal, control diploid hybrids from normal eggs lived to become frogs.

When the denucleated eggs of Bufo communis were fertilized by sperm of Bufo viridis, many eggs began to develop, but died in about fourteen days. The normal, diploid hybrids lived through the metamorphosis.

When the denucleated eggs of Bufo viridis were fertilized with sperm of Bufo communis, the larvae died during gastrulation. The diploid hybrids died at the same stage.

When the denucleated eggs of Bufo communis were fertilized with sperm of Bufo calamita the haploid, hybrid larvae did not go beyond the gastrula stage.

The hybrids between species of Triton are viable, and better results might therefore be expected with denucleated eggs.[3] Paula Hertwig ('23) made experiments with denucleated eggs of Triton taeniatus and the sperm of two other species. When the denucleated eggs of Triton taeniatus were fertilized by their own sperm, haploid larvae were obtained that lived only until they had absorbed their own yolk, i.e., about 22 to 27 days (Fig. 106*b*). There were 12 chromosomes present (haploid) as shown in Fig. 106*d*. When similar denucleated eggs were fertilized by sperms of T. cristatus the haploid hybrid larvae lived only 18 days. They were small embryos with head and tail, with little pigment development (Fig. 106*a*). There were 12 chromosomes present (haploid) as shown in Fig. 106*e*. The normal diploid control (T. taeniatus by T. taeniatus) is shown in Fig. 106*c*. The results were the same when the denucleated eggs of Triton taeniatus were fertilized by the sperms of Triton palmatus, the haploid hybrids developed better, and became pigmented, but lived only 18 days.

These results show that the haploid (arrhenotokous) larvae are weak and are apt to die. Those from the egg and sperm of the same species fare no better than the haploid hybrids. As yet the latter have not been kept long enough to show whether the hybrids, with haploid nucleus of one species and cytoplasm from the other, are more like the paternal than the maternal race. Since the sperm of one species has brought the development of the cytoplasm of the egg of another species to the same stage as sperm of the same species, it may seem probable that haploid

[3] Wolterstorff reports hybrids between Triton cristatus and vulgaris obtained by Poll. Baltzer ('20) had obtained only younger stages from monospermic pieces (obtained by the constriction method) with foreign sperm.

hybrids may yet be obtained. Baltzer has in fact kept one haploid to the time of metamorphosis. Until later stages are obtained, the influence of the sperm of Triton on the cytoplasm of the egg cannot be stated, but genetics has, as I have said, already answered this question.

The fertilization and development of both nucleated and non-nucleated isolated fragments of the egg of Triton taeniatus, and of partially constricted portions of the egg, have been further

FIG. 106.—*a* and *b*, two embryos of Triton that developed from a denucleated egg, fertilized by a spermatozoon; *c*, normal diploid embryo, from control of same age; *d* and *e*, two haploid groups of chromosomes from a haploid larva. (After P. Hertwig.)

investigated by Fankhauser ('24, '25). He confirms the older observations that several sperms usually enter the egg. Of these only one combines with the egg-nucleus to form the segmentation nucleus, the other sperm-nuclei are absorbed without dividing. On the other hand, if a great many sperms enter (more than ten) the cleavage is abnormal, since not only the main sperm-nucleus, but the accessory ones take part in the divisions. Possibly such eggs were not normal before fertilization.

In some of Fankhauser's experiments, the eggs were completely separated by means of a constricting hair into two parts. The fragment with an egg- and a sperm-nucleus develops normally,

but a relatively large number of sperms (more than ten) cause abnormalities. The non-nucleated fragment with several sperm also divides, but the cleavage is abnormal in more than 87 per cent of the fragments, and is delayed. Sometimes all, or most, or only one of the sperms may form spindles.

Fragments of eggs that have been fertilized, but which contain only the egg-nucleus after separation, i.e., fragments without a sperm-nucleus, may later show incomplete furrows which then disappear; or the cleavage may be nearly normal and produce a morula. In one case an embryo formed with closed neural tube.

Fragments of fertilized eggs, without nuclei, may show faint traces of cleavage furrows, but do not divide.

Other eggs were constricted after fertilization until they assumed a dumb-bell shape. A "stalk" or bridge united the two parts. One part will contain the egg-nucleus, and the other will lack it. In some cases both parts may be later supplied with nuclei derived from the main diploid nuclei, as Spemann ('14) had already described. In addition the non-nucleated part may be supplied with sperm-nuclei through the bridge. In some other cases the part containing the egg-nucleus, if it does not contain a sperm, may be supplemented by a nucleus derived from a sperm-nucleus of the other half.

If both parts contain sperms and the bridge is sufficiently narrow, both parts may cleave, due to the independent development of the sperm in the non-nucleated part. If the bridge is broader, the accessory sperm in the non-nucleated part may be suppressed. The inhibition of the accessory sperms decreases with their distance from the bridge. Inhibition of these sperms may also result from the migration of nuclei across the bridge; the new in-wandering nuclei may induce a new cleavage in this part. The independent cleavage of the non-nucleated part is, on the average, less delayed than is that of a separated non-nucleated fragment. This suggests that there is also a speeding up of the sperm induced by the egg-nucleus.

If the non-nucleated part alone contains sperms, the egg-nucleus in one and the sperm-nuclei in the other part, start to develop, but in more than 50 per cent of cases the egg-nucleus unites with one of the sperm-nuclei in the bridge, or in its vicinity. Fankhauser interprets this to mean that there is some sort of action at a distance between the two nuclei. If the two nuclei

do not come together, an independent cleavage begins in the non-nucleated part, seldom at the normal time, more often later. The nucleated part also often shows evidences of cleavage with only the egg-nucleus present, but this takes place only when the egg is much constricted.

The evidence shows that from the main nucleus (diploid), and later from the cleavage nuclei, an inhibiting influence on the accessory nuclei takes place which possibly is due to a chemical substance diffusing into the cytoplasm. There is possibly another interpretation, namely, that in the formation of the chief aster the materials of the egg that form the aster are used up, and, in consequence, delay the progress of the other asters. In contrast to this Fankhauser suggests that the egg-nucleus exerts an accelerating influence on the sperm-nuclei because of a different substance that diffuses from it. It is this influence that makes the nearest-lying sperm-nucleus the chief sperm-nucleus. Possibly here also it is not something diffusing from the egg-nucleus, but the kind of cytoplasm by which it is surrounded that accelerates the nearest sperm.

Conclusions

The study of egg-fragments has brought out certain facts of general interest. The results have shown, for instance, that the mere entrance of the spermatozoön into the egg does not suffice to start development; for, if the egg be cut in two after fertilization, in such a way that the sperm-nucleus is present in one fragment and the egg-nucleus in the other, the former develops but not the latter. It follows that those theories of fertilization that have been proposed which terminate with the entrance of the sperm into the egg are entirely inadequate to explain normal fertilization.

The study of the cleavage of fragments indicates that the pattern is not foreshadowed or predelineated in the protoplasm, but that the form of cleavage appears *pari passu* with the development of the mitotic figure in the piece. The surface conditions of the egg do not appear to condition either the type of the cleavage or the relative sizes of the cells. On the other hand the position and size of the spindles appear to determine the character of the cleavage about to take place. In fact, the propor-

tionate type of the cleavage shown by fragments may be little more than an outward expression of the size of the spindle which in turn is an expression of the material available for its formation. If this inference is correct it calls for a further examination of the phenomenon of gelation (or hardening) of the colloidal material of the egg that appears to be an important part of the physical expression of the mitotic phenomenon. How the alternating changes from gel to sol affect the kind of spindles that develop as cleavage follows cleavage we do not know, and must invite the assistance of the physical chemist to help us forward.

The development of embryos that contain the half number of chromosomes has been definitely established by the study of fragments that contain the sperm-nucleus, and there can be little doubt also but that a fragment containing the egg-nucleus alone, if it could be incited to divide, would also give rise to a haploid embryo.[4] The development of whole eggs brought about by reagents that incite its development prove that one set of maternal chromosomes suffices to produce an embryo. Nevertheless, the delicacy of these haploid embryos is known both from the results of experimental embryology and from genetics. The failure of non-nucleated fragments of the egg of Sphaerechinus to develop into a pluteus under the influence of the sperm of Echinus does not mean that such an occurrence in general is impossible. Boveri has shown, in fact, that a haploid hybrid may be formed in another combination. The question that this experiment was intended to answer has, however, been answered by modern genetics. There is, nevertheless, another question connected with the development of haploid organisms that is most important. It appears that haploid embryos, derived from whole eggs, have cells relatively too large in proportion to the nuclei in them, or, in other words, that the protoplasm does not become adjusted in this respect to the chromatin. The weakness of these embryos is sometimes supposed to be the result of such a maladjustment. On the other hand, a fragment of half size with a haploid nucleus would be expected to have the proper adjustment of cytoplasm to chromatin. It has been supposed that they have, therefore, a better chance of survival. The evidence, however, is at present very fragmentary, and the conclusion doubtful. A similar question arises in those instances where an egg starts its development

[4] See Chapter XXIII on Artificial Fertilization.

with twice the normal number of chromosomes (tetraploid). Geneticists have brought forward several instances of this sort. There is the same cytoplasmic relation in question here, the answer to which may help to solve one of the important problems of development, the quantitative relations of the cytoplasm to the number of chromosomes.

CHAPTER XVI

THE DEVELOPMENT OF A WHOLE EMBRYO FROM AN ISOLATED BLASTOMERE

The first experiments undertaken to study the behavior of isolated blastomeres were those of Chabry in 1887. Although his results did not immediately call forth further work in the same direction, yet it is clear in the light of later developments of the subject that Chabry's pioneer work deserves high rank. On the other hand, Roux's experiment, which consisted of injuring one of the first two blastomeres of a frog's egg, became from the beginning the center of interest (1888–1892); for, Roux himself made this experiment the crucial point of far-reaching philosophical speculations concerning development. The experimental work of Driesch (1892–1900) on the sea-urchin's egg, followed by the work of other investigators on eggs of other marine invertebrates, broadened the discussion, and led to many differences of opinion concerning some of the "fundamental" problems of development. The sea-urchin's egg has been found to be much more favorable than the frog's egg for experiments dealing with the isolation of the blastomeres, but not more so than the eggs of ctenophores, molluscs, ascidians, Amphioxus and Triton. It has been found that the isolated blastomeres of sea-urchins, of certain hydroids, of a nemertean, of Amphioxus, of fish, of Triton and of the frog give rise to whole embryos; while the isolated blastomeres of ctenophores, molluscs and ascidians give rise to half-embryos. Before attempting to discuss whether or not there are any peculiarities in the development of these types that furnish any hint as to why in one case whole development takes place, and, in the other, half-development, it will be necessary to pass in review a few typical instances of each kind, because it will be apparent that the differences between the two methods of development are not so sharp as is generally supposed.

One negative conclusion is obvious, namely, that there is no

relation between the systematic position of the animals, whether "high" or "low" in the scale, and the mode of development of their isolated blastomeres; for there is a hydroid and a vertebrate in one group, and a ctenophore and an ascidian in the other.

The Development of Isolated Blastomeres of the Sea-Urchin

The sea-urchin was the first type in which it was clearly shown that isolated blastomeres produce whole embryos. Driesch ('91) shook apart the blastomeres and followed their development. The method that he used to separate the blastomeres was one that had been earlier made use of by O. and R. Hertwig to obtain fragments. They had found that if the eggs of the sea-urchin are violently shaken a few times in a small tube half-full of sea water, the eggs may be broken into pieces. These pieces soon round up into small spheres, and, if sperm is added, some of the fragments segment. This same method, with modifications, was used by Driesch to separate the first two blastomeres.

If the sea-urchin's eggs are fertilized and, then, after a minute or two are so gently shaken that they are not broken, the fertilization membrane, that has formed at the moment the sperm pierced the surface of the egg, may be removed from the egg. This procedure makes the subsequent separation of the two blastomeres, when the egg has divided, quite easy. This separation is brought about by again shaking the eggs, this time more violently, when at the height of their division. It is still easier to separate the blastomeres if, after the removal of the membrane, the eggs are placed in artificial sea water from which the calcium salts have been omitted (Herbst's method) and left there until the first cleavage is finished. The slightest agitation will then suffice to separate the blastomeres. When such isolated blastomeres are returned to the sea water, their development is normal, as control experiments with whole eggs treated in the same way demonstrate.

The isolated cell continues to divide as though its partner were present. The first division is through the pole, and at right angles to the previous first plane of division (Fig. 107a^1). The second division is equatorial (Fig. 107b^1). At the next division two micromeres are formed at the antipole, while the polar cells divide equally (Fig. 107c^1). The result is a half-sphere open

more or less on one side. But even at this time the cells at the open side tend to close in the opening, and after a few more divisions the closure is completed. A spherical swimming blastula of half-size develops.

The half-cleavage shows that each blastomere contains within itself the factors that determine its cleavage pattern. This is important in so far as it shows that the behavior of each blastomere, during normal cleavage, is not due to the form of the blastomere arising from its close contact with its partner.

The details of the later cleavages have not been followed, but one fact of interest has been found. The isolated blastomere

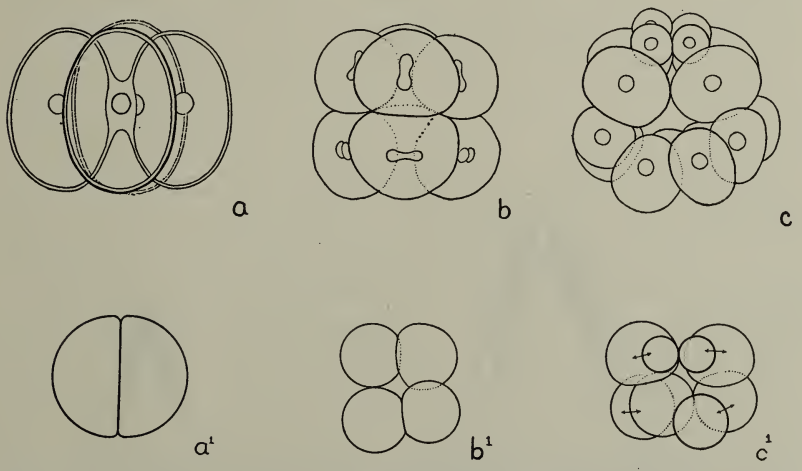


FIG. 107.—*a*, *b* and *c*, four-, eight- and sixteen-cell stages of egg of sea-urchin; *a'*, *b'*, *c'*, first three cleavages of an isolated ½ blastomere. (After Driesch.)

undergoes the same number of divisions that it would have completed had it remained a part of a whole. This means that the number of cell-divisions that take place before the next stage of development is reached is definite, and that the small embryo contains cells of "normal" size, but which are proportionately twice as large as are the cells of the whole embryo. Cell-size is within these limits not a factor that determines the advent of the next step in development.

The mesenchyme cells are produced in the normal way in the half-embryo, but there are only half (or thereabouts) as many as in the normal embryo, and they are proportionately twice too large. Gastrulation is normal (Fig. 108*b*), and the typical tri-

angular stage is soon reached that is followed by a typical pluteus of half size (Fig. 108b^1).

By means of the same methods the four blastomeres of the four cell stage may be isolated. Each divides as though a part

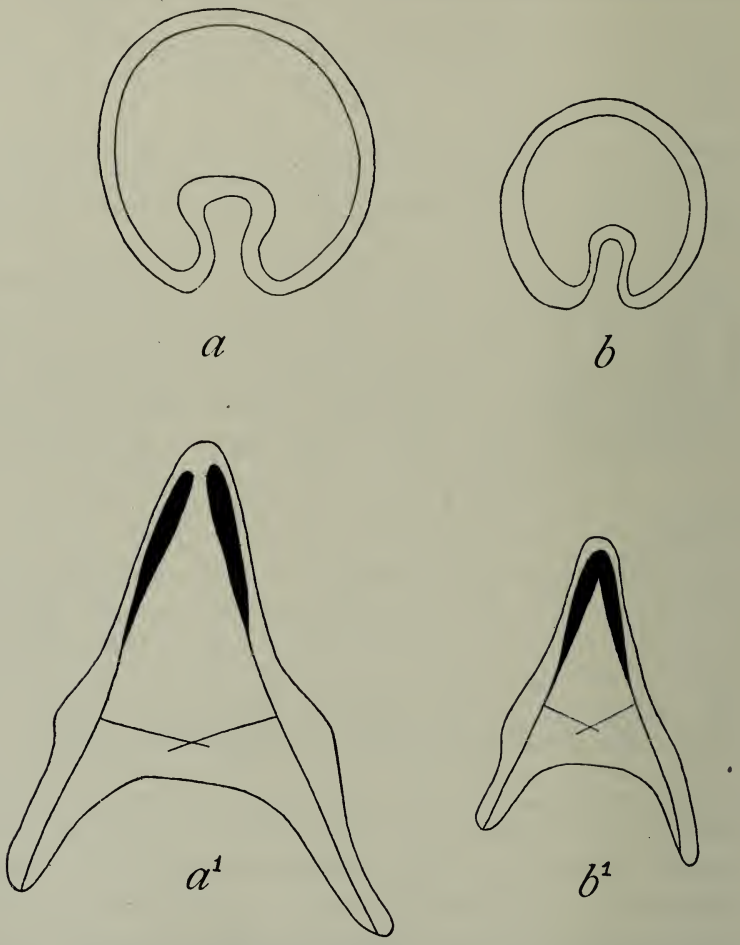


Fig. 108.—Whole and ½ gastrulae and plutei of sea-urchin; the latter from isolated ½ blastomeres.

of the whole, forming one micromere at the ¼ sixteen-cell stage. A spherical, free-swimming blastula stage follows, which gastrulates in some cases, and a small imperfect pluteus may be formed. Some of the ¼ blastulae never develop beyond this stage. Many of them stop their development in the gastrula stage,

and the plutei in other cases are, as a rule, imperfectly formed. Since each ¼ blastomere is supposed to contain samples of all the materials of the egg, this frequent failure to develop normally cannot be attributed to absence of any special kind of material. One is inclined to attribute the failure to the smaller sphere with its different surface tension relations, or to some incompatibility between the size of the cells and the size of the sphere. If, as seems to be the case, the cells are of the same size as those of a corresponding stage in the whole embryo, they are proportionately four times too large in the smaller ¼ embryo.

If, in the eight-cell stage, the cells are separated, a ⅛ polar blastomere gives a somewhat different result from a ⅛ antipolar blastomere. Each kind segments as a part, and each kind may produce a gastrula with a tripartite gut and a rudimentary pair of spicules. Most individuals, however, go no further than the swimming blastula.

The polar ⅛ blastomere frequently produces a blastula covered by relatively long cilia that are of the same length as those in the normal gastrula. The blastula from the antipolar ⅛ blastomere more often dies than does the former kind.

The fate of the isolated blastomere of the 16-cell stage depends on the region from which it comes. If it is one of the micromeres, it divides only a few times, forming a heap of about 10 cells. These soon die. A 1/16 mesomere may produce a blastula with long cilia, and sometimes an imperfect gastrula that may have a rudiment of a skeleton. A macromere (middle-tier cell) may develop into a gastrula with mesenchyme and spicules. The original differences in sizes would seem responsible, in part, for these results, but only in part; for, the outcome appears also to be due to differences present in the different regions.

It is probable that some at least of the 1/32 blastomeres will occasionally show the beginning of gastrulation.

How far the failure of the smaller blastomeres to produce later stages is due to regional difference, and how far to size alone is difficult to decide from this evidence—both conditions may be involved. More significant are Driesch's ('00, '02) experiments in which groups of blastomeres from polar and antipolar regions were studied. These results will be described below.

The three outstanding features of these experiments with isolated ½ blastomeres of the sea-urchin's egg are, first, that the

cleavage is partial; second, that normal embryos usually result, and when they do not do so the failure does not appear to be entirely connected with regional differences separated by cleavage but to other conditions present; and third, that whole or nearly whole development, may take place with cells *relatively* two times, or four times, or even eight times larger than those present in the normal embryo.

Isolated Groups of Cells of Later Stages

Driesch ('00, '02) has systematically isolated groups of cells after the third cleavage. When the four cells of the polar hemisphere ("animal half") are isolated, only hollow, ciliated blastulae with ciliated ring and stomadaeum develop in the majority of cases, but a few gastrulate and form normal (?) larvae. When the four cells of the antipolar hemisphere ("vegetative" or gastrula half) are isolated, most of them die, but those that live gastrulate, and may form normal (?) larvae. The result must be interpreted to mean that, before the time of the third division, differential cleavages of some sort have taken place in the egg, possibly materials have been separated more or less completely that represent the outer and inner cells of the future embryo. There is some uncertainty in the normal embryo as to the number of cells that form the mesenchyme together with those that are invaginated to form the archenteron. If half of the cells are turned in as Boveri thought, then the plane of separation must be nearly in the plane of the third cleavage—hence even the cells derived from the four polar cells will be near or in the region of endoderm cells. If, however, as others suppose, the number of cells turned in is not so great, the four polar cells will be outside of this region, and in those individuals that gastrulate the endoderm will contain cells that would normally form only ectoderm. It may seem more probable that there is no such sharp distinction at this stage, but that cells in the equatorial region may be potent to form either part according to the region in which they come to lie later.

It was supposed that the mesenchyme cells that give rise to the skeleton are derived from the micromeres. Driesch ('02) removed the four micromeres, and found that the remaining cells produced a normal embryo with skeleton. It seems to follow

either that cells in the neighborhood of the micromere group are also capable of becoming mesenchyme (in the absence of the micromeres), or else that some of these cells may normally contribute to the mesenchyme. The isolated micromeres themselves form only a group of indifferent cells.

After the fourth cleavage there are sixteen cells present of which four (in the antipolar hemisphere) are the largest and are called macromeres. They are, taken together, a little less than ½ of the volume of the egg. These cells contribute to the endoderm of the embryo according to Boveri. When isolated these four cells form a small gastrula with mesenchyme and small triradiate spicules. Whether the archenteron is proportionately too large in them is uncertain. Since there is an outer shell of ectoderm it follows that these cells are capable of producing this layer—whether they do so normally is not definitely known. The presence of the spicules confirms the other results given above in showing that cells other than the micromeres may exceptionally (?) produce these structures.

The eight polar cells of the 16-cell stage were also isolated. They constitute about ½ of the volume of the egg. The outcome is somewhat the same as when the four cells of the 8-cell stage are isolated. For the most part they produce only blastulae with long cilia, only a few gastrulate with mesenchyme, and triradial spicules.

Driesch ('95) has also shown that when free-swimming blastulae are cut in two with small scissors, most of the pieces as large as half the whole blastula will gastrulate and form normal (?) larvae. Since the cutting was at random, some at least of the pieces may be supposed to represent the polar hemisphere, others are antipolar. Still others—the majority—will have been cut obliquely. The operation is, however, too crude to permit of definite conclusions except in so far as the results show that there must still remain in the blastula stage a considerable power of "self-regulation." If the blastulae are cut in two *after* the gastrulation process has begun, the halves from the polar hemisphere ("animal" halves) do not gastrulate or form mesenchyme, etc., although they may form a ciliated ring and stomodaeum. Whether these halves still correspond to the halves of earlier stages is doubtful; for, it is probable that at the time when the gastrulation begins the cells of the antipolar hemisphere have already

started to concentrate at the antipole, and that the ectoderm cells have correspondingly flattened and cover a larger area of the surface. Hence it is uncertain from these results whether the cells of the polar hemisphere have really lost their power to form endoderm, or whether these halves represent regions that previously lay higher up in the egg. It is quite possible that both changes may have taken place at this time, i.e., that the "upper" cells have already begun to differentiate as ectoderm and that the "lower" cells have moved down further into the antipolar hemisphere. If so, the results are inconclusive.

The Development of Isolated Blastomeres of Hydroids and Medusae

The development of isolated blastomeres of several species of hydroids was studied by Zoja ('95). The eggs were found to be too delicate to be broken into fragments by shaking, but it was found possible to cut apart the blastomeres without injuring them by means of a needle with a knife edge.

The blastomeres were first cut apart at the two-cell stage to give ½ blastomeres. After the next division some of the half-blastomeres were again separated to give ¼ blastomeres. When these had divided, some of them were again separated to give ⅛ blastomeres. As Zoja points out, it is conceivable that the smaller blastomeres (¼ and ⅛) obtained in this way might give a different result from those obtained by separating the ¼ blastomeres when the normal egg had reached that stage. Comparison of the results of the two methods showed, however, that the outcome is the same.

The ½ blastomere of Clytia flavidula continues to segment as though in contact with the other half, except that the cells very soon arrange themselves into a complete sphere (Figs. 109*a-e*). A swimming, hollow blastula develops and from this a normal hydroid, which is very little, if any, smaller than the normal hydroid. The ¼ blastomere goes through similar changes (Figs. 110*a-c*). The ⅛ blastomere also forms a hollow blastula (Figs. 111*a-e*). It slowly becomes filled with endoderm, but does not develop further. The 1/16 blastomere segments, and forms first a hollow then a solid swimming planula that does not pass beyond this conditon. Another hydroid, Laodice cruciata, gives essentially the same results.

The hydro-medusa Liriope mucronata, has a different method of forming its endoderm. After the fifth cleavage, when the 32 cells form the thick-walled blastula, a division occurs so that 32 inner endodermal cells are cut off (delaminated) from the 32 outer cells (Fig. 112*a-c*). The former then join each other to

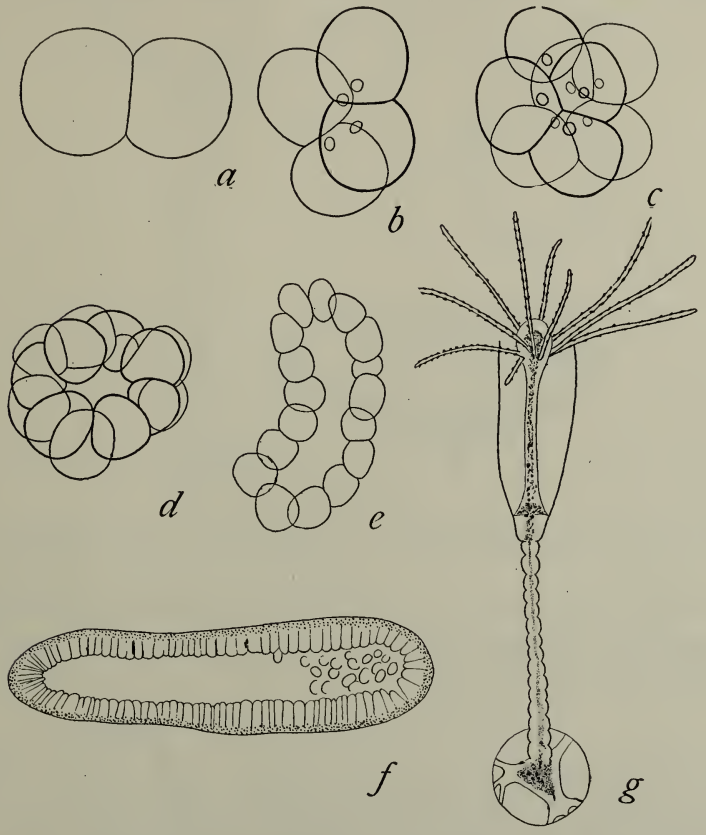


Fig. 109.—*a–c*, cleavage and development of ½ blastomere of egg of hydroid, Clytia; *d–e*, cleavage and development of ½ blastomere. (After Zoja.)

make an inner hollow sphere, the endoderm. Later the inner and outer spheres fuse at one point of the surface, and the mouth of the jelly-fish opens at this point. The isolated ½ blastula (112*d*, *e*), forms its endoderm after its fourth cleavage (16 cells). Later it produces a small jelly-fish with four tentacles. The ¼ blastomere segments and delaminates, forming a two-layered embryo that does not develop further.

Zoja's results are similar, in all essential respects, to those from isolated blastomeres of sea-urchin eggs. The isolated blastomeres segment as though still in contact with their fellows,

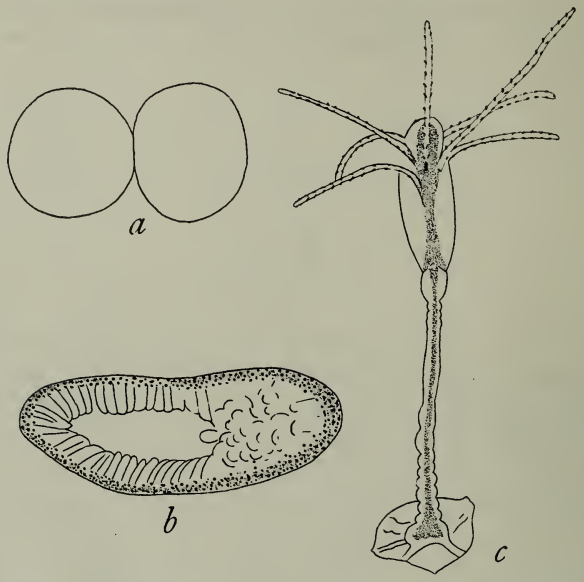


Fig. 110.—Cleavage and development of ¼ blastomere of egg of hydroid, Clytia. (After Zoja.)

although, in the absence of peculiar cleavage patterns, this relation is not so apparent. The ½ and ¼ blastomeres may form typical embryos (hydroids or jelly-fish), but the ¼ cells some-

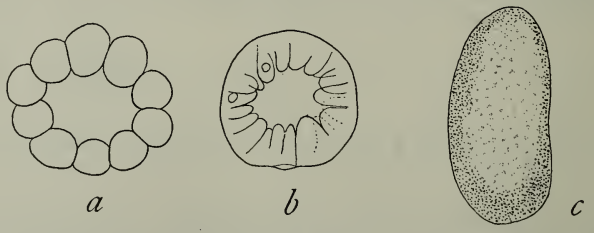


Fig. 111.—Blastula and embryo of ⅛ blastomere of egg of hydroid, Clytia. (After Zoja.)

times fail to reach this stage, and the ⅛ cells never form hydroids.[1]

[1] Maas ('01), has made a few isolation experiments with hydroids' eggs, and Conklin ('08), with the eggs of a hydroid, Linerges.

The fertilized eggs of the scyphomedusa, Renilla, have been cut into pieces by Wilson ('03). In the normal egg the segmentation nucleus divides from three to four times before the cytoplasm divides. The first division of the fragment is also into 8 to 16 blastomeres, and at the same time as the normal control (after 24 hours). Many of these fragments produce swimming dwarf larvae, which later sink to the bottom, develop tentacles, and

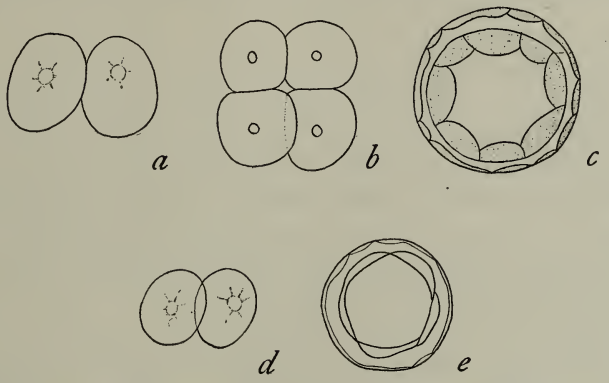


Fig. 112.—Cleavage and development of ½ blastomere of egg of jellyfish, Liriope. (After Zoja.)

produce a first pair of buds which, as in the normal embryos, are the beginning of colony formation.

The Development of Isolated Blastomeres of Amphioxus

Normal Cleavage and Gastrulation

The cleavage of the egg of Amphioxus has been studied by numerous embryologists, Kowalevsky ('67, '77), Hatschek ('81, '84), E. B. Wilson ('93), Morgan and Hazen ('00), MacBride ('98), Cerfontaine ('06–'07), Conklin ('24). One of the recent and most detailed accounts is that of Cerfontaine. He has discovered certain facts about the early bilaterality of the fertilized egg and early cleavage stages that were overlooked, or only partially described, by earlier observers. Since Cerfontaine's description supplies the information most needed for present purposes it will be closely adhered to in the following account.

The eggs pass through the early stages in the ovaries or gonads that lie on each side of the atrium of the female. When fully

formed, the eggs pass into a secondary ovarian chamber. In this chamber the large egg-nucleus disappears, the chromosomes condense and come to lie on the first maturation spindle. There seem to be present about a dozen chromosomes on the spindle in the form of tetrads. The first polar body is given off. It lies later outside of the membrane of the egg, which means that a membrane develops after its extrusion. A second polar spindle is then formed. The eggs are set free in this condition in the late afternoon.

If the adults are collected at this time and placed in dishes of sea water, they begin almost at once to liberate their eggs. At the right season of the year many females will deposit their eggs on certain days and then for several days no eggs, or very few, are laid by newly caught females. A few days later eggs can again be obtained in abundance, etc. The causes of this rhythm are not known, but no external conditions have as yet been discovered that are responsible for it.

The eggs pass from the secondary ovarian chamber into the peribranchial space (through openings in the wall of each gonad) by the contraction of the muscles of the body wall. From the peribranchial space the eggs pass out of the atriopore to the exterior.

A second membrane has been formed when the egg was set free from the ovary beneath the first membrane already present. The new membrane comes from a substance derived from the surface of the egg beneath the first formed membrane. This substance hardens on its outer surface to form a thin envelope, within which a large amount of fluid soon collects. In this fluid the egg floats. The second polar body is then set free. It remains in contact with the surface of the egg and serves throughout the cleavage stages to mark the pole of the egg.

The sperm enters the egg near the antipole. Its head forms a nucleus that moves toward the polar hemisphere. As the egg- and sperm-nuclei enlarge and come together they are found to lie not in the primary axis of the egg, but somewhat to one side. Their position indicates the first appearance of a bilateral arrangement of the fertilized egg. Not only is the bilaterality indicated by the position of the spindle, that develops about the united nuclei, but also by the distribution of the yolk granules and protoplasm that have now a slightly different arrangement

in what becomes later the anterior and posterior sides of the egg. Cerfontaine believes that the bilaterality of the egg is present even prior to the extrusion of the second polar body. The region richer in protoplasm comes, he thinks, from the area occupied by the large egg-nucleus. But if this area lies in the primary axis, as appears to be the case, its excentric position only becomes evident after the conjugating pronuclei have moved out of that axis. Hence the bilaterality can be present only after this movement has taken place. In the light of the evidence from the ascidian egg, it seems probable that the bilaterality of the egg of Amphioxus is present only after fertilization when the pronuclei have moved to one side of the primary axis. Whether they have moved to a predetermined side of the egg, or whether this side is determined by the excentric entrance of the sperm cannot be made out from the evidence.

The first cleavage is in the plane of bilateral symmetry and divides the egg into equal parts (Fig. 113*a*). The second division

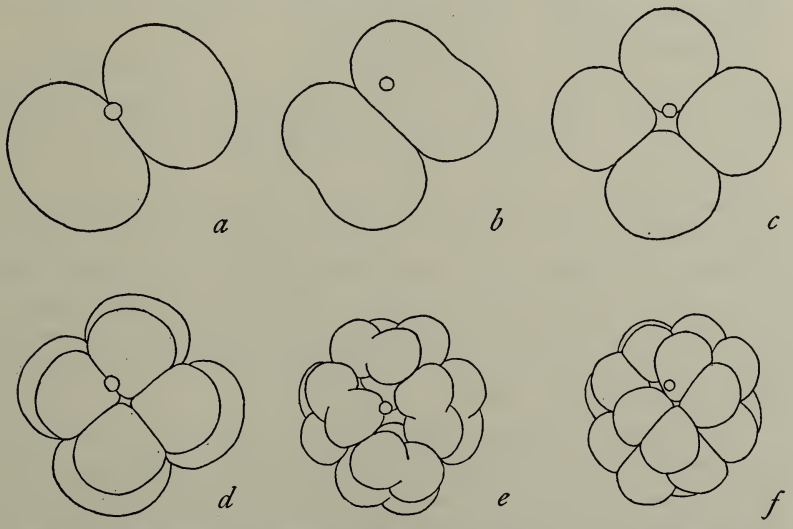


Fig. 113.—First four cleavages of the egg of Amphioxus. (After Cerfontaine.)

is slightly unequal (Fig. 113*b*, *c*). The two smaller blastomeres are on the antero-dorsal side, the two larger blastomeres are on the postero-ventral side. The third cleavage (Fig. 113*d*) is at right angles to the two preceding ones and is unequal. Four smaller apical blastomeres and four larger basal blastomeres are

formed. The former stand more or less symmetrically over their larger sister blastomeres. Not infrequently there is some variability in the relative position of these cells. In fact Wilson distinguishes several types of cleavage with all intermediate conditions. Cerfontaine is more inclined to treat these variations as slight abnormalities due to the artificial treatment the eggs have received.

The divisions of the eight cells, to reach the next stage, are not quite synchronous. The divisions of the cells on the antero-dorsal side are a little more advanced than the divisions of those on the opposite side. The divisions of the four smaller cells begin in a meridional plane, as judged from the location of the spindles, but change a little as the cells constrict, so that finally the plane is more nearly at right angles to the first plane of division (Fig. 113*e*, *f*). The four larger cells divide in a slightly oblique plane owing to the presence of a cavity in the midst of the four larger cells. The cleavage is unequal. The four smaller cells are in contact with the cells of the polar hemisphere, the four larger cells lie around the antipole.

In the next cleavage the eight cells in the polar hemisphere divide equally, those of the other hemisphere somewhat unequally. At the end of the division there are eight tiers of four cells each, whose dimensions increase slightly in each tier from the pole to the antipole. Four cells lie around the pole and four around the antipole. The cells on the antero-dorsal side have shown a tendency to divide a little sooner than those on the opposite side. These cells are also a little smaller—a difference that is traceable to the four cell stage. The cleavages that give 64 cells, and 128 cells and even 256 cells have been followed, but they offer nothing of special interest.

The egg, or embryo begins now, to flatten at the antipole preparatory to gastrulation before 256 cells are present. Even at the 128 cell stage the cells have begun to flatten against each other to form a sort of epithelium or blastula wall. The segmentation cavity had first appeared at the four cell stage in part as a consequence of the spherical shape of the blastomeres. In the following stages of cleavage it increases in size and may be open at the pole and antipole as late as the 16 or 32 cell stage.

The gastrulation of Amphioxus has been studied by several embryologists (Kowalevsky, Hatschek, Lwoff, Sabotta, Klaatsch,

MacBride, Samassa, Morgan and Hazen). All agree in regard to the main point, viz., that the lower hemisphere is turned in, to form a cup-shaped gastrula (Figs. 58*c*; 114*b*), whose opening (blastopore) diminishes in size until it is reduced to a small pore near the posterior end of the embryo. But there is much difference of opinion concerning the relation of the primary axis of the egg to the gastrula axis, and of the latter to the long axis of the embryo; also concerning the method of closure of the blastopore and concerning the kind of cells that come to line the interior of the cup. Without attempting to discuss these questions it will suffice to follow Cerfontaine in his conclusions that are on the whole in harmony with those of several other observers.

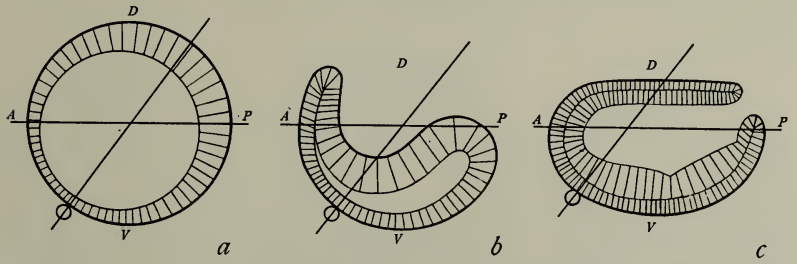


FIG. 114.—Diagram showing the relation of the primary axis of the egg of Amphioxus to the gastrula axis. (After Cerfontaine.)

The relationship of the axes of the egg, gastrula and embryo are indicated in the diagram (Fig. 114*a-c*). The changes in shape that bring about the transition from *b* to *c*, Fig. 114, are difficult to make out. They seem to be due in large part to the actual bending over of the anterior end of the embryo, but this alone would not close the blastopore unless accompanied by other changes. Some of these may be brought about by the turning in of cells around the rim of the blastopore, especially at the anterior margin. The cells must also shift on each other at this time so that fewer lie at the rim in later than in earlier stages. Cerfontaine thinks that during closure the right and left sides of the blastopore are brought together (concrescence), and this, taking place from before backwards, may account fully or in part for the advance of the dorsal lip.

The cells that line the "roof" of the archenteron (dorsal side) are smaller than those of the sides and floor, and contain less yolk. These cells are very much like the "ectoderm" cells that

lie at first outside the rim of the blastopore. Those at the anterior end of the blastopore are turned in when the invagination first takes place, and additions are probably made to them as the lateral lips are brought together. There has been much discussion as to whether these cells are to be called ectoderm or endoderm, but this discussion has lost interest since we have come to pay more attention to the cell-lineage of the embryo than to phylogenetic questions based on imaginary two-layered ancestors of these stages.

When the blastopore has become reduced to a pore, the dorsal surface anterior to it flattens and then assumes the form of a wide groove. On each side the ectoderm pushes toward the middle line, especially from behind forward. It arches over the blastopore

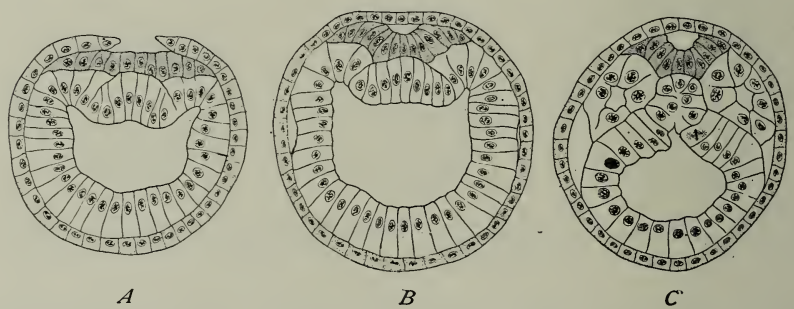


Fig. 115.—Cross-sections of embryos of Amphioxus, showing the formation of the neural plate and tube, notochord, and gut-pouches. (After Cerfontaine.)

as a roof. The cavity beneath the plate of ectoderm so formed remains open at the anterior end for some time, and is called the neuropore. The dorsal plate of ectoderm that has been over-arched now begins to roll in from the sides to form a tube, the central nervous system of the embryo. Meanwhile changes have also been taking place in the dorsal wall of the archenteron. These changes can best be shown by cross-sections through the middle of the embryo (Fig. 115).

Along the mid-dorsal line of the archenteron a narrow band of cells—about 8 to 10 in cross-section—begins to round up to form a chord. On each side of this chord the cavity of the archenteron is constricted as a result of the sinking of the dorsal wall (Fig. 115), so that a groove is left along each dorso-lateral side of the archenteron on each side of the notochord. Simul-

taneously with the rounding up of the notochord, the cells of the archenteron along these lateral grooves draw together to form hollow pouches, each retaining at first its communication with the archenteron. These pouches are the protosomites (mesoblastic somites). They are paired left and right. The first to form are near the anterior end. The pouches continue to deepen and to separate from the archenteron from before backwards. Later the cavity of each is lost. The inner wall of each applies itself to the nervous system and notochord to form the connective tissue sheath around the notochord and the muscles on each side. Other mesodermal parts also develop later, from the protosomites. In front of the first pair of protosomites a pair of rather broader pouches is given off, right and left, from the archenteron, one of which becomes the head cavity and the other the club-shaped gland.

The Development of Isolated Blastomeres of Amphioxus

The blastomeres of the egg of Amphioxus are easily shaken apart if placed in tubes half-filled with water and then gently shaken a few times. Many eggs fall apart into their component blastomeres. Often the cells are only partially separated, and then twin embryos arise.

As Wilson ('93) has shown the isolated ½ blastomere retains for a short time its original form slightly flattened on one side. It soon becomes more rounded and divides into equal parts, the cleavage plane being at right angles to the original first cleavage, i.e., it is in the same position as in the normal egg. In many cases the next cleavage is unequal (as in the corresponding normal cleavage), although sometimes equal (Fig. 116*c*). At the next division, four smaller cells appear (micromeres). Wilson interpreted this stage as a duplication of the whole normal eight-cell stage rather than a half-cleavage type.

A symmetrical whole gastrula is soon formed, and a normal embryo of half size develops (Fig. 117*b*, b^1).

"The ¼ blastomere may likewise segment quite like the entire ovum (Figs. 116*e*, *f*) but the unequal type of the four celled stage is more frequent than in the ½ embryo. . . ." Many of these ¼ embryos gastrulate but relatively few form embryos of one fourth the whole size.

The one-eighth blastomeres of which there should be at least two types, divide at first into equal parts, and then into unequal

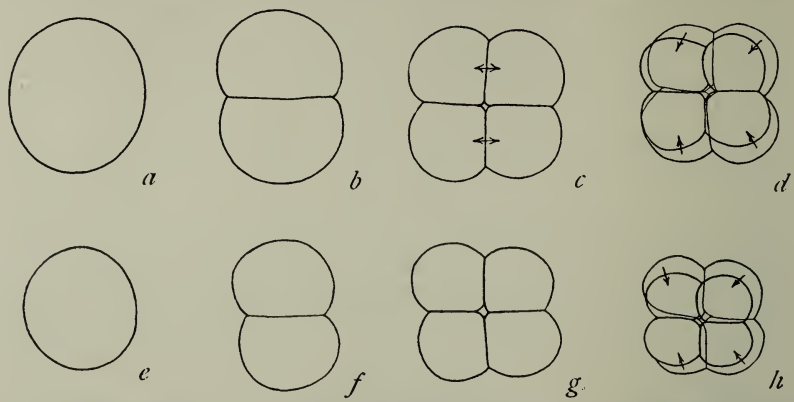


Fig. 116.—Cleavage of the ½ isolated blastomere, and of the ¼ isolated blastomere of Amphioxus. (After Wilson.)

parts (Fig. 118*a*). At the next division "four micromeres are formed as usual by an equatorial cleavage, but these may be equal or unequal." As the cleavage continues a large cleavage pore

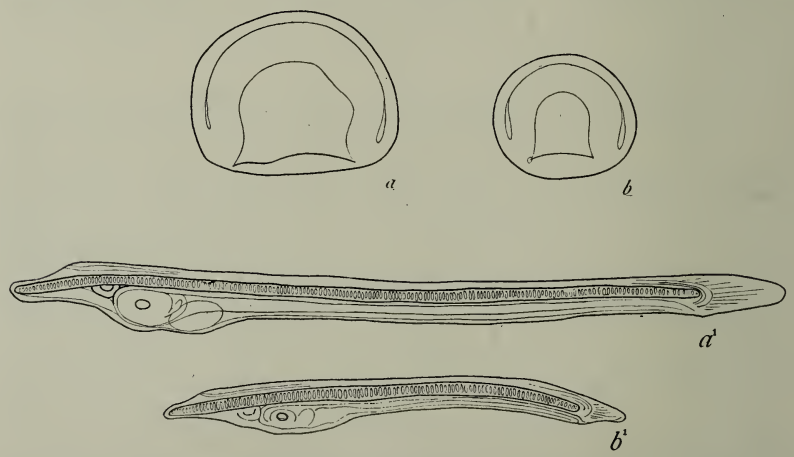


Fig. 117.—*a*, normal gastrula, and *b*, ½ gastrula of Amphioxus; *a'*, normal, and *b'*, ½ embryo of Amphioxus. (After Wilson.)

appears. Bent or even flat plates of cells are later formed (Fig. 118*e*, *f*). A few half-closed blastulae were found, or, more rarely still a closed blastula (Fig. 118*g*). None of these gastrulated.

These ⅛ embryos may swim about for several days and then die.

It is clear from the account that a great deal of variation in the cleavage of the isolated blastomeres exists, and this makes it difficult to determine whether they segment as parts or as miniature wholes. Wilson is more inclined to interpret the cleavage as a whole cleavage, but in the light of the great variation recorded for whole eggs and isolated cells, and in the light of the many other cases of partial cleavage that have since been described it is uncertain what interpretation to put on these results. In any case there is no doubt that whole gastrulae and whole embryos develop.

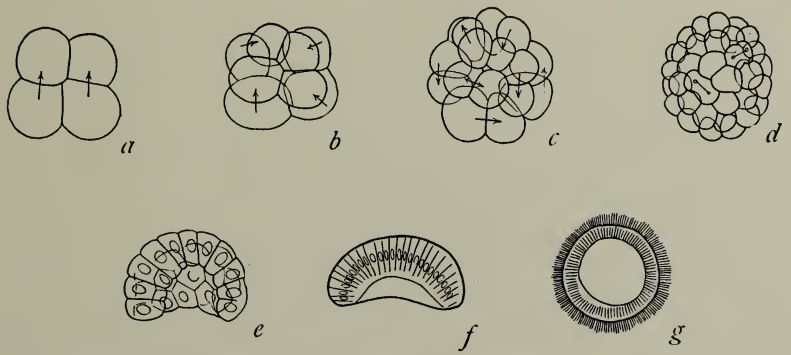


Fig. 118.—Cleavage of ⅛ blastomere of Amphioxus. (After Wilson.)

The question may be raised whether both of the ½ blastomeres have the same value, i.e., whether each may equally develop into a whole larva. This could only be determined by following both blastomeres from the same egg, and this has not yet been done. In another way, however, some information has been gained. When the blastomeres are only partially separated twin-gastrulae develop, as Wilson's figures abundantly demonstrate, but he states that in only one case did he obtain two whole embryos or twins, and the figure shows that one of them is deficient in the head region. If, as Cerfontaine shows, the first two blastomeres correspond to the right and left halves of the normal embryo there is good reason to suppose that both blastomeres have the same value, and, in general, such an interpretation is in agreement with Wilson's results. Wilson points out that "a few of the one-half larvae at this stage (stage of the first gill-slit) are perfectly developed in every respect, but as the figures show, the normal

proportions of the body are not quite maintained, the regions behind the expanded anterior part being relatively shorter. The notochord is relatively thicker, its diameter being nearly or quite equal to that of the normal larva."

Whether all the isolated ¼ blastomeres have the same potency may seem more doubtful in the light of Cerfontaine's evidence, since two of them are normally more anterior-forming cells and two more posterior-forming cells. That all may gastrulate seems improbable [2]—some of them at least do so; but a later larval stage is rarely attained, and none normally constituted were observed. In a single isolated instance a ¼ larva was obtained at a stage nearly corresponding to that of the first gill-slit. This larva possessed a nearly normal notochord, neural tube and neuropore, mesoblastic somites and preoral pit, but had no mouth, no gill-slit, no anus, and the posterior region of the intestine was aborted.

These results with Amphioxus leave little doubt but that the development of the isolated ½ blastomeres of Amphioxous conforms closely to that of the sea-urchin, fish and frog type. A few further details concerning the composition of the ½ and ¼ larvae were made out by Morgan ('96, '01). The ½ and ¼ blastomeres can be shaken into pieces and the pieces with nuclei will develop, as Wilson also had observed. In this way it was found possible to get information concerning the failure of the one-eighth blastomere to develop beyond the blastula stage. It was found that nucleated pieces of ½ and ¼ blastomeres, as small in volume as the ⅛ blastomere, do not develop beyond the blastula stage, although larger fragments may do so. It seems possible, therefore, that the failure of some of the ⅛ blastomeres to pass beyond the blastula stage, may be due to a progressive change in the direction of differentiation that they have undergone, but also to a deficiency in size. The proof is not complete, for other factors may be involved in the two cases.

It is a question of some interest whether the ½ and ¼ whole larvae of Amphioxus are made up of the same number of cells as the normal embryo, which would then be only half as large as the normal cells, or whether the number is only a half or

[2] Conklin has more recently stated (General Cytology. 1924) that he has been unable to get whole development from every individual blastomere of the 4-cell stage.

a fourth as many and the cells are full size. There are several practical difficulties in the way of determining this question, but the result seems fairly certain, namely, that the half-larvae have half as many cells as the whole larvae, and the quarter larvae have one fourth as many (Morgan). The cells in both are of normal size. It would seem to follow that within these limits the size of the cells is not a determining factor in organ formation, but nevertheless the results show that this general relation does not hold for all the organs. A calculation of the number of cells was made by cutting into sections, whole, ½ and ¼ larvae of approximately the same age. Since the smaller larvae develop more slowly, it is not easy to identify corresponding stages. Moreover, since the sections were of the same thickness and not proportionate to the size of the larvae, it is necessary for purposes of comparison to take sections through the same structural region, rather than to compare sections that have the same number from the anterior end. The cross-sections of the ½ larvae are expected to show about two-thirds the total number of cells, and the ¼ larvae one-half, if the total number of cells present is respectively one-half and one-fourth. This is true, in general, but apparently not for certain organs. For instance the notochord and nerve cord appear to have about as many cells as have the same structures in the same regions of the normal larvae. These cells must be obtained at the expense of the ectoderm and endoderm respectively which would be correspondingly diminished, but this loss, if real, would be too slight to be detected in these two germ-layers. In fact the cross-sections of the ectoderm and of the digestive tract show about the number of cells expected for cells of full size, namely, in cross-section ⅔ and ½ respectively of the number in the whole larva.

The Development of Isolated Blastomeres of Cerebratulus

Wilson ('03), Yatsu ('04), and Zeleny ('04) have given a very complete account of the cleavage and development of isolated blastomeres of Cerebratulus and their results are in agreement on all points.[3]

When the two blastomeres are cut apart each tends to round

[3] The normal cleavage of Cerebratulus has been sufficiently described in an earlier chapter (Fig. 82), in connection with the behavior of egg fragments.

up into a sphere, and resembles an egg of half-size, yet the cleavage is strictly partial, even as to the sequences of the spiral divisions, showing, once more, that the form of cleavage is intrinsic, and not a function of the shapes of the blastomeres due to their apposition.

The isolated ½ blastomere first divides in a plane passing through the pole, i.e., in the same plane in which it would have divided had the egg remained intact (Fig. 119*b*). The next cleavage (Fig. 119*c*) in each cell is unequal—a larger polar cell

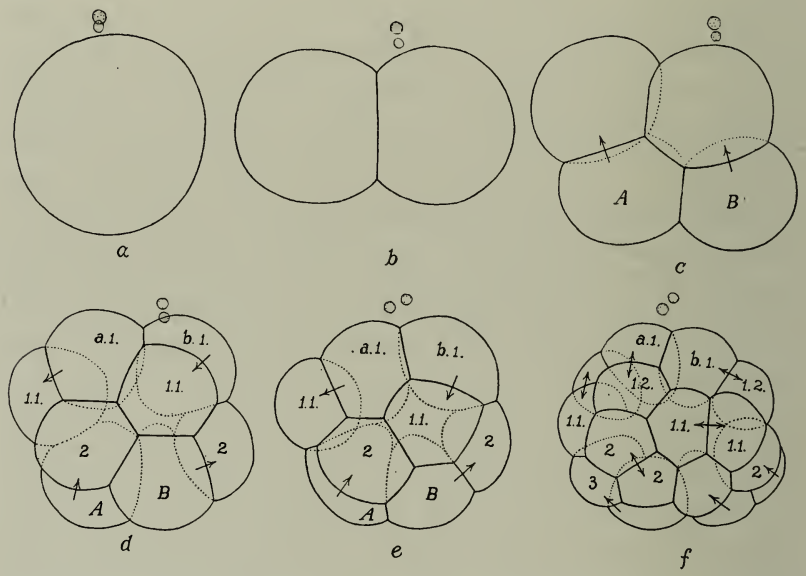


Fig. 119.—Cleavage of ½ isolated blastomere of egg of Cerebratulus. (After Wilson.)

and a slightly smaller antipolar cell result. The direction of the division is leiotropic. The third division is dexiotropic (Fig. 119*d*, *e*), the planes of division being the same as in the normal egg for each half. The individual cells are, however, more rounded than the corresponding cells when the other half is present. On one side there is still a wide opening into the segmentation cavity. As the cleavage advances this opening at the side closes, and a spherical blastula of half-size results. Symmetrical gastrulae and swimming larvae or "pilidia" (Fig. 121*a*, *b*) develop from these half-blastomeres,

The ¼ blastomere divides at first into a larger polar cell and a slightly smaller antipolar one. The next cleavage is leitropic producing one-fourth of a normal 16-cell stage (Fig. 120*b*). The third cleavage is dexiotropic. The opening at the side closes even earlier than in the ½ blastulae. Some of these embryos gastrulate, although many fail to reach this stage. Pilidia-like larvae develop in some cases (Fig. 121*c*, *d*).

Yatsu obtained similar results. A few further details from his work may be added. At the two-cell stage the blastomeres were either cut apart, or shaken apart in Ca-free water. Forty pilidia were obtained; some were defective, the rest perfect. The apical organ was sometimes defective or double. From the ¼ blastomeres, 21 pilidia developed. One lacked the apical organ,

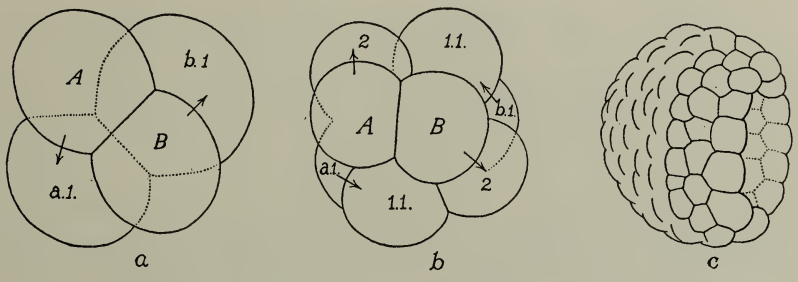


Fig. 120.—Cleavage of ¼ isolated blastomere of Cerebratulus; *a* and *b*, closed type of cleavage, owing to shifting of cells; *c*, open blastula stage of Cerebratulus. (After Wilson.)

one had no enteron, and the remaining 19 were more or less abnormal, the commonest malformation being the shifting of the apical organ to one side. In general appearance these embryos strikingly resemble young pilidia. "The ¼ embryo shows in every respect features of arrested development such as were seen, though in less degree, in the ½ pilidia."

At the 8-cell stage the embryo was divided by a vertical cut (in the primary axis) into two parts. In four cases perfect pilidia developed. In another experiment, the upper quartet (A) of the 8-cell stage was separated from the lower quartet (B). Seven embryos were produced from the former (A) and three from the latter (B). From the A-set, one nearly round pilidium was produced, one had a solid central endoderm, and five lacked the archenteron. From the B-set one nearly normal pilidium and

two very defective embryos were obtained. These results when compared with those from "vertical" cuts show that vertical halves are more likely to develop into normal pilidia than are horizontal halves, although some nearly normal embryos may come even from the latter. Since in both cases the isolated pieces were halves, the difference in the results cannot be due to a difference in size of the pieces or to the number of cells contained in them.

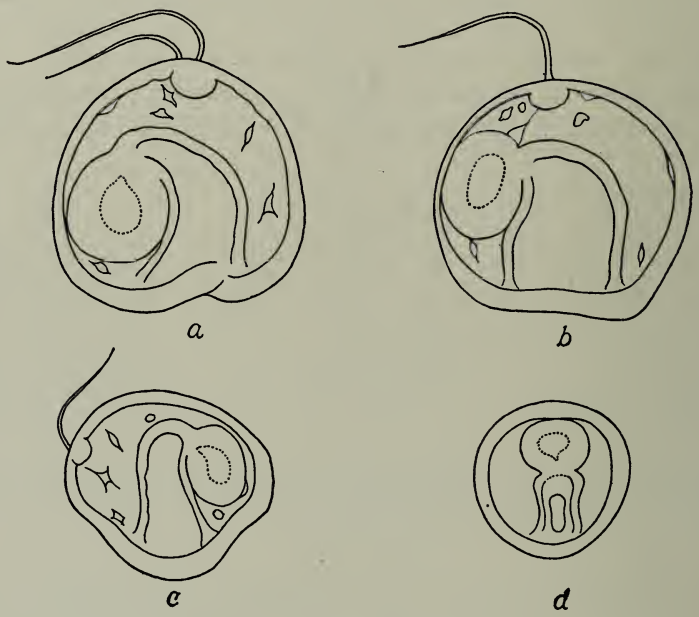


FIG. 121.—*a* and *b*, twin pilidia from isolated ½ blastomeres of same egg; *c*, pilidium from isolated ¼ blastomere; *d*, pilidium from same, imperfectly developed. (After Wilson.)

The blastula of Cerebratulus can be cut into two with a knife, and each half will close its open side and develop further. Operations of this kind have been carried out by Wilson, Yatsu and Zeleny. Wilson cut embryos from 12 to 24 hours old (128 + cells). When cut vertically (i.e., through the primary axis) the halves give rise to asymmetrical larvae, each with an apical organ and a closed archenteron "but the former is situated at one end of the somewhat elongate body, while the archenteron is strongly bent toward the same end."

When the blastula is cut in two at the equator, the halves may produce pilidia, but these often show characteristic defects. The pilidia from the upper (polar) half always develop the apical organ, but the archenteron is relatively small; while the pilidia from the lower (antipolar) half may lack the apical organ, but may have an abnormally large gut. These results seem to mean that even in the late blastula stages the specialization has so little developed that cells from the equator may form an archenteron and also an apical organ. Operations on gastrulae and young pilidia were also carried out by Yatsu. The apical region, with a considerable portion of the upper hemisphere, was cut off. Three of the remaining pieces failed to develop an apical organ but four developed it.

The Development of Single Blastomeres of Fish

The fish's egg has quite a different distribution of materials from that of the other eggs so far considered. When fully formed the egg is made up of a central sphere of yolk, covered by an envelope of transparent protoplasm free from yolk. This layer is in close contact with a firm membrane around the egg. At the pole there is a minute canal through which the spermatozoön enters. Fertilization takes place normally in sea water within a minute or two after the eggs are laid. Contact with sea water induces changes in the protoplasm. It begins to collect in a flat disc beneath the micropyle. While it is "flowing" into this region the two polar bodies are given off; the head of the sperm, now within the egg, swells up to form the sperm pronucleus, which then unites with the egg pronucleus. The segmentation nucleus lies near the middle of the disc. The surface protoplasm which is at first in close contact with the membrane soon becomes free from it, and the egg rotates within the membrane. If the egg is undisturbed, the disc may remain under the micropyle, but if the egg is rolled about it may shift its position. In pelagic eggs, that float at the surface of the water, the disc (and usually the micropyle) is turned downward since this is the heaviest part of the egg. In other eggs, especially those that adhere to a solid surface, the disc may be uppermost.

Segmentation begins about an hour after fertilization. The cleavage divides the disc into equal parts (Fig. 46*b*), but does

not pass quite through it, nor does it pass around the egg (Fig. 122). The second cleavages are at right angles to the first, cutting the disc again so that four equal blastomeres are formed (Fig. 46*c*). Each cell rests upon the yolk beneath and is continuous with it. The yolk is, therefore, continuous at this time with all the cells of the disc. At the periphery of the disc the cells are also continuous with the thin layer of protoplasm over the egg (Fig. 122*b*, *c*). The third cleavage in each cell is either parallel to the first or makes an angle with it, and in extreme cases it may be parallel to the second division in one or more

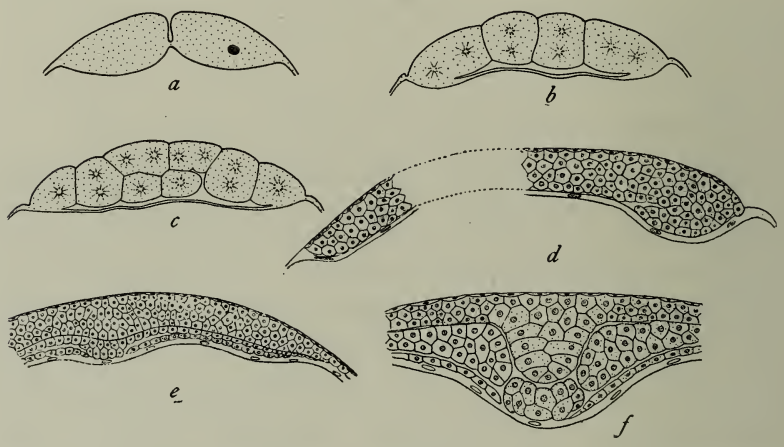


FIG. 122.—*a*, *b*, and *c*, sections of cleavage stages of teleost fish; *d*, median section through young blastoderm, showing turning in of tongue of cells at posterior end; *e*, cross-section of older stage, showing thickening of outer layer cells to form the neural plate and formation of mesoderm and notochord beneath, also the endodermal plate; *f*, enlarged view of cross-section of older stage, showing nerve cord, notochord, mesodermal plates and endoderm. (After Wilson.)

quadrants. The eight cells are about equal in size (Fig. 46*d*, *e*). As the cleavage of the disc continues, some of the cells divide horizontally so that the disc becomes two or more layers deep (Fig. 122*c*). The blastoderm begins to flatten on the yolk. Some of the deeper lying mitotic spindles send one daughter nucleus into the yolk. In consequence the upper layer of the yolk beneath the disc comes to contain a layer of nuclei embedded in a continuous (syncytial) layer of protoplasm containing yolk. The margin of the disc thickens and a flange of cells turns in under the disc (Fig. 122*d*, *e*). At one part of the disc (the posterior

region) this flange is thicker (Fig. 122*d*), and extends further inwards until later it reaches the center of the disc. The disc now begins to extend over the yolk, soon reaching the equator (Fig. 123*c*, *d*), then more rapidly closing over the opposite hemisphere, the rim continually getting smaller until it finally closes near the antipole.

When the equator is reached the outer layer of cells, above the thickest part of the inturned flange, begins to grow thicker and narrower from side to side, and this process becomes more marked as the rim extends below the equator. The material of this ridge becomes the solid neural cord. It is thickest in front where the brain develops. Here also a pair of solid outgrowths form the beginning of the eye vesicles. The tongue of invaginated cells gives rise to the plate of endoderm and notochord, and on each side to a layer of cells that forms the mesoderm.

While the blastoderm is travelling over the yolk it becomes stretched so that it becomes thinner, except where the neural plate is forming. The material of the rim, called the germ-ring, moves toward the axis to become incorporated in the embryo by a sort of "concrescence." The incorporation of a part of the germ-ring in the posterior end of the embryo has been shown by cutting the ring with a needle on one side (Fig. 123*e*). This leads to a slight deficiency of materials on that side, both of ectoderm and of mesoderm (Morgan '95, Kopsch '96).

If the newly laid eggs are placed in a centrifuge, or roughly handled, the central mass may be moved, so that the center of the disc no longer coincides with the micropyle. This is most easily done after fertilization. If shifted before fertilization, the first sperm that enters may lie at some distance from the disc that contains the egg-nucleus. A small accumulation of protoplasm appears about it and then begins to travel up towards the disc, where the two nuclei may unite. If they fail to unite, each nucleus appears to become an independent center, and two discs result that may later fuse into one.

If the rotation of the disc takes place after fertilization, a second sperm may enter through the micropyle. It becomes surrounded by a small amount of protoplasm to form a small secondary disc. This disc may fail to fuse with the large disc, and a few divisions may take place in it as it lies on the side of the egg. Presumably the sperm-nucleus in such a disc is haploid.

These small discs fail to produce embryos. The cause of the movement of the protoplasm toward the sperm-nucleus has not been explained. The movement of the sperm discs toward the

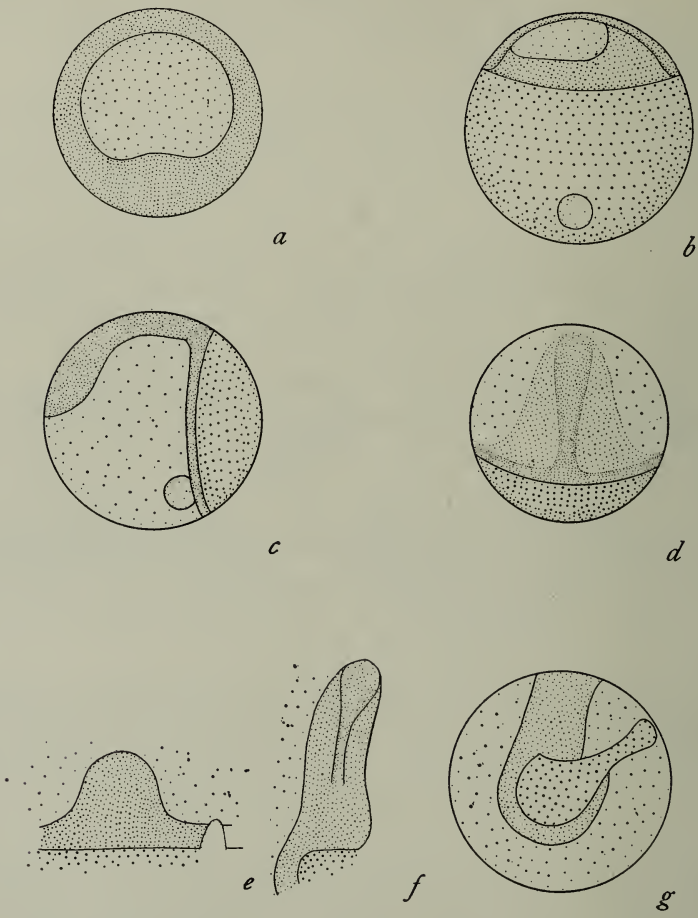


Fig. 123.—*a*, blastoderm of fish, the thickening at the posterior rim indicates where the embryo will be formed; *b*, side view of egg in same stage; *c*, side view of older stage, showing embryo and germ-ring; *d*, same stage, showing embryo, the nerve cord, and germ-ring; *e*, part of the germ-ring of a young embryo that has been cut on the right side; *f*, older stage of same; *g*, older stage at time when germ-ring is closing in. (*a–d*, after H. V. Wilson and *e–g*, after Morgan.)

central mass may, however, be only a part of the more general movement of the surface protoplasm.

It is not possible to isolate the first two blastomeres, but the

same end is obtained by puncturing, with a needle, one of the two cells. The material of the punctured cell exudes when the needle is withdrawn, or can be pressed out through the opening. The remaining cell will then continue to divide, as has been shown by Morgan ('93, '95). It lies on a sphere of yolk that is relatively twice too large for it, but since, at this time, all the protoplasm has not passed into the region of the disc, the embryo will be in reality something more than half the size of the whole embryo.

The ½ blastomere rounds out to form a nearly circular disc. It segments into two, then into four cells, etc. (Fig. 124). The

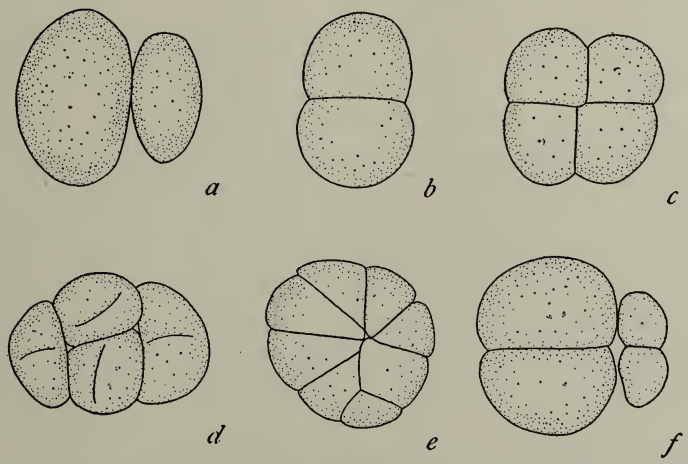


FIG. 124.—Two-cell stage, showing unequal first division of egg of fish—the smaller cell was removed; the later cleavage of the remaining blastomere is shown in *b–e*. (After Morgan.)

subsequent changes are the same as in the normal egg. A small "whole" embryo on a large yolk results.

If three of the first four cells are destroyed, the ¼ blastomere that remains may form a ¼ embryo. A reservation must, however, be made in the case of the ¼ embryos. The evidence goes no further than to show that in some cases such an embryo develops. It has not been proven that each of the first four blastomeres has the same potentiality.

Experiments have been made both on the small pelagic egg of Ctenolabrus and on the relatively large egg of Fundulus. The results in both show that whole embryos are formed from ½ and ¼ blastomeres. There are no indications of partial structures.

The factors that determine the position of the embryonic axis in the fish egg have not been made out. Observations of Clapp ('91) and of Morgan ('93) show clearly that the axis of the fish embryo does not as a rule coincide with the first plane of cleavage, but may make any angle with that plane. Observations of the disc and of the early blastoderm have not as yet shown any regional differences that determine the location of the embryo. The bilateral symmetry of the ½ embryo also indicates that there is no necessary connection between the first cleavage plane and the embryonic axis.

Two-headed fish embryos have often been described. It appears that they arise, when, simultaneously at two points on the rim of the disc, a thickening occurs, each of which begins to differentiate as the head-end of an embryo. As the materials of the rim draw together, especially after the equator of the egg is passed, the two embryonic regions come together and a single body results. If the heads start near together they are the sooner united, and a fish with two heads is formed; but, if they arise far apart, they may develop independently for a longer time, and two embryos result united at the middle, or even in the posterior end of the body. These double embryos have been sometimes supposed to be due to double fertilization, but this is certainly not the case, because in all instances in which double fertilization has been observed (as indicated by the type of cleavage that results) the egg never forms any embryo at all. In cases where the disc is moved away from the micropyle and in which more than one sperm enters, normal embryos may develop, but not double ones so far as observed.

An estimation of the number of cells in the half-embryos has been made by Morgan ('95). The results are not entirely decisive, and the reasons for this are not far to seek. There is great variability in the length of different ½ embryos. The ½ embryo has the whole of the yolk, over which to extend, as has the whole embryo, since the cytoplasm of one blastomere but no yolk is removed. On the other hand, all of the protoplasm out of which the embryo is developed has not passed into the first two cells at the time of operation. The periphery of the blastoderm continues to receive accessions from the superficial protoplasm throughout all the early cleavage stages. It is, therefore, not certain that the cells, derived from the blastomere that remains, may not receive

some of the protoplasm that was destined to go into the removed blastomere (or its products). Moreover, since the head-end of the embryo is first laid down it is quite possible that the organs of the head may actually make use of more than half of the material present at the time, to the detriment of the posterior parts of the embryo, that develop later. The results as stated above are not entirely convincing, but they seem to show that, in the anterior region at least, the cells in the eyes and nervous system are nearly as numerous in the ½ embryo as in the normal embryo. Since most of the embryos are shorter (and those that are full length are narrower) than the normal, the total number of cells appears to be approximately half, and presumably full-size, or not far from it.

Herlitzka ('97) found that in the ½ embryo of Triton the nervous system and notochord have the same size that they have in the normal embryo, while the intestine and myotomes of the ½ embryo are smaller in cross-section. He found that the number of cells in the cross-section of the nerve-cord is the same in the one-half and whole embryos, but in the myotomes the number in the ½ embryo is just half that of the whole embryo. It is not certain, however, whether all these half-embryos came from "anterior" halves, or from right or left blastomeres. There may possibly be some differences in the two kinds of embryos.

Isolated Blastomeres of Triton

The blastomeres of Triton taeniatus have been isolated, after freeing them from the vitelline membrane, by Gudrun Ruud ('25). When taken out of their inner membrane just before or during the first cleavage, it is possible to separate the first two blastomeres completely by laying a glass rod in the furrow (Fig. 125). Each half may be again separated in the same way, if ¼ blastomeres are to be studied. The results are, as was to be expected, the same as when the first two blastomeres are separated by a constricting thread or hair. Two types of ½ embryos result. When the first cleavage is in the prospective median plane, two whole embryos develop, one from each blastomere. When the first cleavage is frontal, one whole embryo results from that blastomere containing the prospective dorsal lip, and one imperfect gastrula-like form from the other "ventral" blastomere.

The upper four blastomeres ("animal half") were also separated from the lower four ("vegetative half") in eggs in which the third cleavage was in a horizontal plane (equatorial). The upper four produced only a mass of cells (hyperblastulae) with an irregular contour due to active growth and spreading of the cells. In a few cases a blastopore appeared, but these arise only when one or more of the upper four cells extends down into the vegetative field, presumably into the region of the "dorsal" side.

The lower four blastomeres are often inhibited in their develop-

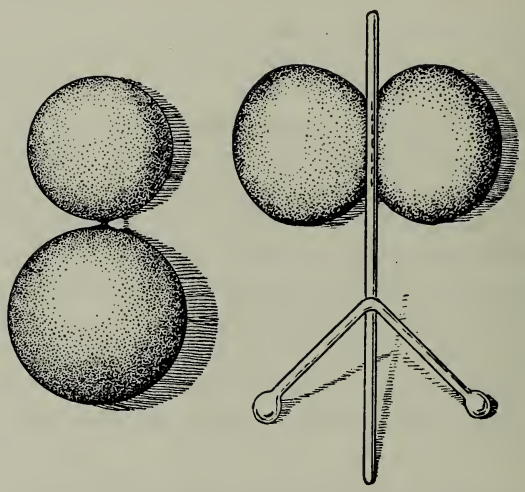


FIG. 125.—Two-cell stage of egg of Triton that has been removed from its membranes (to left). The two blastomeres are separated by means of a glass rod (as shown in figure to right). (After Mangold.)

ment but in those that survive, gastrulate and develop a neural tube, the embryo is practically a normal one.

The isolated ¼ blastomeres from one egg are expected to be of two kinds, according as to whether they come from the "dorsal" side or the "ventral" side of the egg. This is true irrespective of whether the first cleavage was median or frontal. In all, only eleven reached the neural stage. In ten of these, two whole ¼ embryos were obtained from the same egg. Never more than two whole ¼ embryos were obtained from one egg. The other two blastomeres of the same egg developed into hyperblastulae. In one case only one neurula developed; the other three embryos were hyperblastulae. Here it is probable that the first

cleavage had been at an angle of 45 degrees, and only one of the first four blastulae contained the dorsal region of the egg.

These and a few other observations of Miss Ruud are in accord with other experiments on the egg of Triton, and demonstrate that the totipotence of the isolated blastomeres is in direct relation to the presence of the materials of the dorsal side, that corresponds to the crescent region of the frog's egg.

CHAPTER XVII

THE DEVELOPMENT OF PARTIAL EMBRYOS FROM AN ISOLATED BLASTOMERE

The fertilization and cleavage of the Ascidian egg has been studied by Kowalevsky ('66, '71), Van Beneden and Julin ('84), Seeliger ('85), Chabry ('87), Hill ('90), Castle ('96), Crampton ('97), Conklin ('05), and others. The most recent and detailed account is that given by Conklin ('05) of Styela (Cynthia) partita and this account is followed here unless otherwise stated. The eggs when fully formed fall from the wall of the ovary into its cavity and accumulate in the oviduct. The egg is enclosed in a tough membrane over whose outer walls there is a layer of follicle cells. The surface of the egg consists of a broad envelope of protoplasm in which are embedded peculiar test-cells, that have been derived at an early stage from the tissues of the ovary. They are later expelled from the egg, and take no part in its development. Yolk-granules fill the interior of the egg. A large nucleus is present, at first, at or near the center of the egg.

When the nuclear wall breaks down, either before or after the egg is laid, the nuclear sap mixes with the surrounding protoplasm to form a "clear substance" that moves up to the pole and spreads out as a cap. The maturation spindle lies in this clear material, just below the surface (Fig. 28*a*). The spindle has blunt poles without centrosomes and is without astral radiations. The first polar body is not given off until the spermatozoön enters.

The spermatozoa pass between the follicle cells, through the underlying membrane, and one fertilizes the egg, usually within a few minutes. The successful spermatozoön always enters near, or at, the basal pole. The moment the spermatozoön enters, an extensive series of changes begins in the egg. These changes are most strikingly seen in the living eggs of Styela, where, owing to the yellow pigment granules in the peripheral protoplasm, the movements of the egg-substance can be followed. Similar changes

may also be seen in the eggs of Ciona. Almost immediately after the entrance of the spermatozoön some of the peripheral layer of protoplasm (which is nearly uniformly thick), and the great mass of nuclearplasm (in which the maturation spindle lies) flow over the surface of the egg towards the lower pole, leaving the first maturation spindle surrounded by only a small amount of protoplasm. Thus, within some ten minutes after the entrance of the sperm, the protoplasmic pole of the egg is transformed into a sort of yolk-pole and the antipole becomes the more protoplasmic pole. The peripheral protoplasm is filled with yellow pigment granules, and these are carried with the surface layer to the lower hemisphere where they collect, forming there a yellow spot, which surrounds the sperm-nucleus (Fig. 28*c*). The "clear" protoplasm, that came to the surface at the pole, also flows down and comes to lie beneath the yellow disc; it is also visible around the periphery of this disc. The yellow material then gradually spreads out until it covers most of the antipolar hemisphere.

The sperm-nucleus next begins to move towards the equator of the egg to meet there the egg-nucleus. As it moves, a large part of the yellow protoplasm goes with it, and finally arranges itself as a yellow band or crescent (Fig. 28*c*), in whose middle the sperm-nucleus lies. The middle of the crescent marks the later posterior end of the embryo. Its horns reach half-way around the egg to the right and to the left.

While the yellow crescent is forming, the "clear" protoplasm, which surrounds the sperm-nucleus and its aster, also moves from the antipole to the future posterior side of the egg. Finally, when the sperm- and egg-nuclei have met near the posterior side of the egg these two nuclei with their surrounding "clear" protoplasm, move inward to the center of the egg, while the yellow protoplasm is left mostly at the surface.

When the yellow and the "clear" protoplasm flow toward the antipole, the grey-colored yolk is left exposed at the pole. Later when the yellow protoplasm moves upward to the equator, the yolk is left exposed over the entire egg, except for the area of the yellow crescent and a narrow line of "clear" protoplasm that comes to the surface just above the crescent.

It has been stated that the sperm penetrates the egg near or at the antipole. It moves in a radial direction until it reaches the yolk. Its path up to this point is called the penetration

path. Its aster, derived from the middle piece of the spermatozoön, lies behind the sperm-nucleus. They next rotate, so that the aster is directed forward in the later movement of the nucleus and aster to the equator. This path is called the conjugation path. Conklin states that the conjugation path is not always the most direct route to the equator, but, in some cases the sperm-nucleus may take even the longest path to the equator, i.e., across the axis of the egg. Since its final position marks the later posterior end of the embryo, and fixes the bilateral plane of the egg, through which the first cleavage passes, it becomes a matter of importance to know whether the final position is determined by a bilateral structure present at this time, or even earlier, in the egg. As pointed out by Conklin, if the sperm-nucleus took the shortest path to the equator, and the entrance point was accidental, it would follow that the median plane of the embryo was not predetermined, but determined by the point of entrance of the sperm. Since the sperm-nucleus does not take this course, but may take any path to the equator, and fix, thereby, the future course of events, Conklin is inclined to conclude that there exists in the egg a bilateral structure that determines in some unknown way that the sperm-nucleus moves into its plane.

It does not seem to me that this conclusion is inevitable from the facts for, we do not know how often the sperm takes the shortest path to the equator and how often it departs from it. Moreover, since the penetration path is towards the egg-nucleus, the path may be determined in part by the position of that nucleus after the extrusion of the second polar body. If it is assumed that the second polar body is not given off at the exact pole of the egg—in fact there is no good reason for assuming the pole to be a fixed spot—the starting point for the egg-nucleus may be, as just stated, a factor that sometimes causes the sperm to deviate from the shortest path to the equator. Again attention may be called to the statement that while at first the sperm-nucleus occupies the center of the yellow protoplasm, this substance spreads out a little later and occupies nearly the whole lower hemisphere before the sperm begins to travel to the equator. We do not know whether the center of the hemisphere of yellow protoplasm lies at the antipole or in the sperm-nucleus or somewhere nearby. Its distribution may be another determining factor in the direction of the penetration path of the sperm. At least until

these questions are definitely settled I doubt if the evidence furnished establishes the conclusion that there is a bilateral structure to the egg that regulates the direction of the penetration path.

There is one further piece of evidence bearing on the situation. Rarely two sperm may enter. Both enter near the antipole and "at first they lie in a common protoplasmic field. As they move toward the equator, however, they frequently separate, and when they have reached the equator, and have each given rise to a spindle they are often found on opposite sides of the egg with the surrounding protoplasmic fields quite separate." One of them fuses with the egg-nucleus. The spindles in the two masses place themselves parallel to each other, so that a single cleavage plane might pass through the equator of both spindles and through the pole of the egg. Unfortunately these eggs never cleave, so that we cannot tell whether one or two mid-planes are present or where they or it may lie. Conklin concludes that the evidence from dispermy shows that the point of entrance of the sperm is not predetermined, and he concludes also that the results render probable the view that the plane of bilateral symmetry is not first established by the accidental path of the spermatozoön within the egg, but that the plane is structurally present before fertilization. This latter conclusion does not seem to me to be made any more probable by these facts than is the opposite conclusion, namely, that the penetration path is not predetermined by a bilateral structure of the egg, for the evidence shows that each sperm passes to a different point at the equator.

While variations in the cleavage pattern of Styela appear to be very rare, in another ascidian, Ascidiella aspersa, studied by Chabry, many variations occur. These Chabry has described in much detail. He found that, in nearly all cases, such eggs produce abnormal embryos. In general, the earlier in the cleavage the variation occurs the more abnormal is the embryo.

The results of an alteration in the cleavage planes of the egg of another ascidian, Ciona intestinalis, brought about by compression (Morgan '10) are in harmony with Chabry's results. The embryos that develop from such eggs are abnormal—the degree of their abnormality corresponding to the stage at which the change takes place—the earlier the alteration the more abnormal the embryo.

Conklin describes the first cleavage of the egg of Styela as occurring about forty minutes after fertilization. It passes through the pole and through the middle of the yellow crescent (Fig. 126*a*, *b*), dividing the egg into exactly equal halves. The second cleavage (Fig. 126*c*) occurs thirty minutes after the first cleavage. It is at right angles to the first one and also passes through

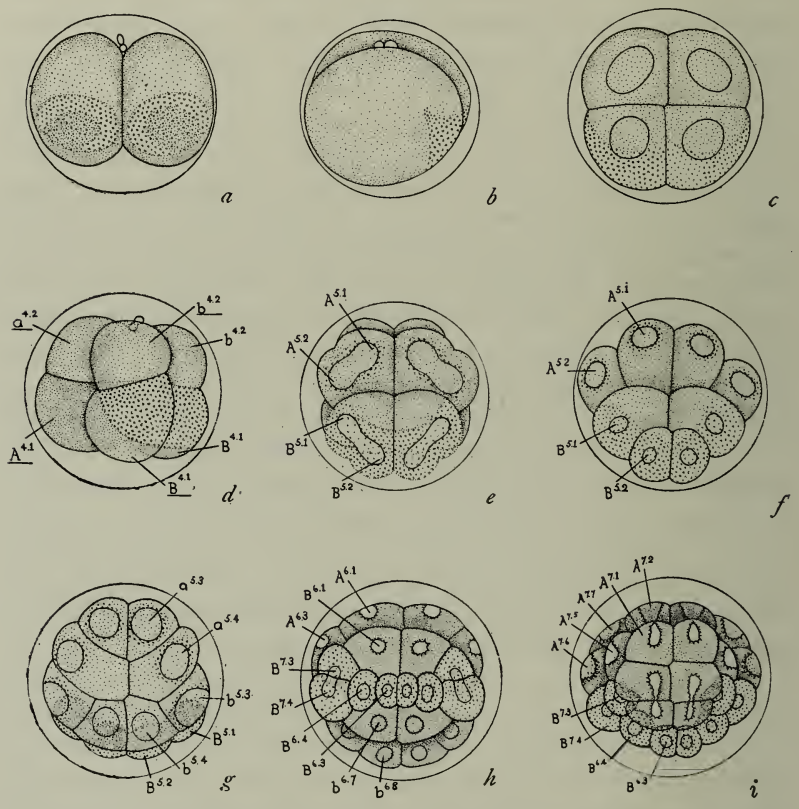


Fig. 126.—Cleavage stages of the egg of Styela (Cynthia) partita. (After Conklin.)

the pole. The two posterior cells (*B* and B) contain nearly all of the yellow crescent and are slightly larger than the two anterior cells (*A* and A). The two posterior cells contain about the same amount of clear protoplasm as the two anterior ones, but contain less yolk.

The third cleavage (Fig. 126*d*) occurs thirty minutes after

the second. It lies nearly in the third dimension of space. Four smaller apical (upper) cells, and four larger antipolar or basal (lower) cells result. The four upper cells slant somewhat forward, i.e., the four polar cells lie slightly anterior to the four lower ones. The disparity in size between the upper and lower cells is most marked in the anterior cells (Fig. 126*d*). The anterior

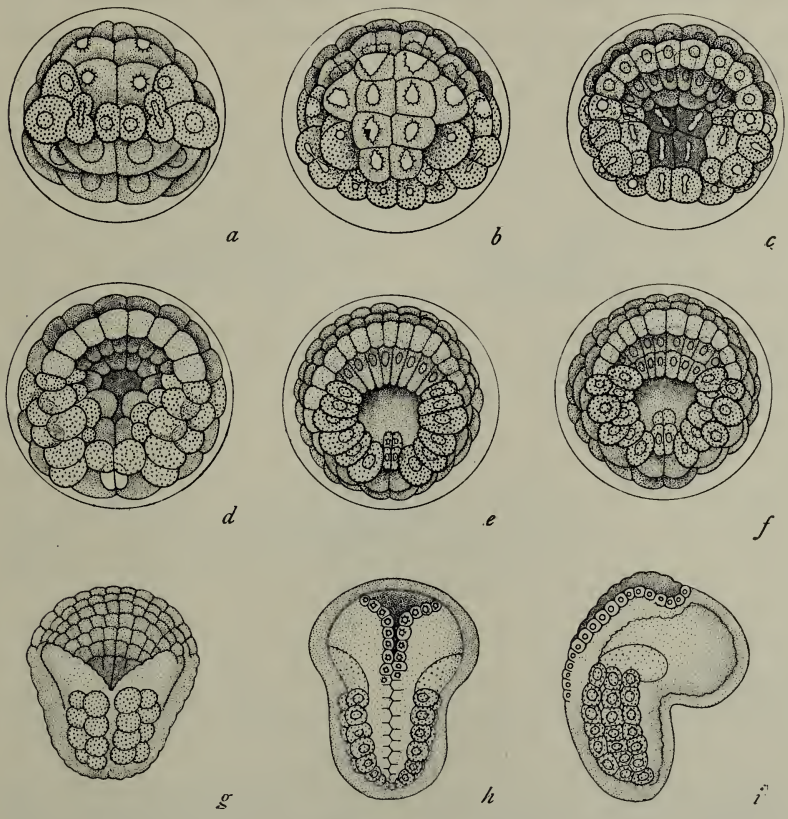


FIG. 127.—Later cleavages, gastrulation, and formation of embryo of Styela. (After Conklin.)

dorsal cells ($A^{4.1}$) are the largest. The anterior ventral cells ($a^{4.2}$) are the smallest. The posterior dorsal cells ($B^{4.1}$) are very little larger than the posterior ventral cells ($b^{4.2}$). The "clear" substance is present in all eight cells, but more abundant in the four ventral ones; the yellow crescent is confined almost entirely to the two posterior dorsal cells; yolk is present in all

the cells, but most abundant in the two anterior dorsal cells ($A^{4.1}$).

As regards the future of these eight cells, the four ventral cells (*a*, a, *b*, b) give rise only to ectoderm, and the four dorsal cells (*A*, A, *B*, B) give rise to endoderm and mesoderm, except that four of the neural plate (ectoderm) cells ($A^{7.4}$, $A^{7.8}$) will come from the anterior portion of the dorsal hemisphere at the 44-cell stage. The mesoderm and endoderm are first completely separated at the 22-cell stage.

The fourth cleavages (giving 16 cells, Fig. 126*e*, *f*) are approximately horizontal and oblique to the median (the first) and transverse (the second) planes. All the divisions are into approximately equal cells except that of the posterior dorsal cells, $B^{4.1}$, and $B^{4.1}$. These cells divide very unequally producing two very small posterior cells $B^{5.2}$ and $B^{5.2}$ that serve from this time forward as important landmarks (Fig. 126*f*). The details of the later cleavages can be understood only by means of numerous figures showing different points of view. The essential facts are given in the following tables.

TABLE XXII

	5th Cleavage, 32 Cells	6th Cleavage, 64 Cells	6th and 7th Cleavage, 76 Cells	6th and 7th Cleavage, 112 Cells	6th, 7th and 8th Cleavage, 132 Cells	6th, 7th and 8th Cleavage, 218 Cells
Polar Hemisphere						
Ectoderm	14	26	26	52	52	104
Neural	2	6	6	12	12	24
Antipolar Hemisphere						
Endoderm	6	10	10	10	20	26
Chorda	.....	4	8	8	8	16
Neural	4	4	8	8	8	16
Mesenchyme	.....	8	6	14	20	20
Muscle	6	6	12	8	12	12
Total	32	64	76	112	132	218

It will be noticed that, beginning with the seventh cleavage, some of the cells divide before the others, thus after the 64-cell

stage there is a 76-cell stage (because some of the 64 cells have not yet divided), which is then followed by the 112-cell stage, and this by the 132-cell stage, etc.

At the last stage recorded in the table (218 cells) the basal (lower) field has sunken in to form the gastrula (Fig. 127*a*, *c*; 128*a*). The six pairs (12 cells) of yellow muscle-cells lie on each side of the blastopore, and are still uncovered by the ectoderm. The notochord comes from 16 cells in two rows of 8 each, that are covered by the neural plate. The 16 cells of the neural plate

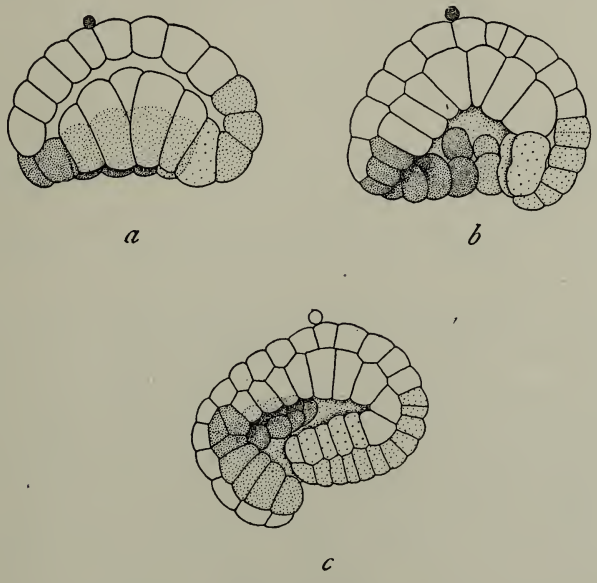


Fig. 128.—Normal gastrulation process of ascidian, as seen in optical section in side view. (After Conklin.)

that cover the notochord, and form the anterior rim of the blastopore, belong to the upper (dorsal) hemisphere. The rest of the neural plate is composed of cells from the basal (ventral) hemisphere. They are arranged in four rows of 6 each, and lie partly in front of the chorda region. The polar body, still attached, is situated posterior to the middle of the ventral surface.

The closure of the blastopore takes place more rapidly at the anterior than at the posterior side, the latter remaining nearly stationary (Fig. 128). The posterior border of the blastopore is formed of mesoderm cells derived from the crescent. These

cells become rolled in at the lateral margins of the blastopore as it closes. They are carried in by the final closure of the blastopore, that takes place mainly from the sides and from the posterior end.

The Development of Isolated Blastomeres of the Ascidian Egg

Chabry ('87) has given a detailed account of the cleavage and development of single blastomeres of the egg of Ascidiella aspersa after the destruction of one or more of the blastomeres by puncture with a glass needle. He found that the remaining blastomere produces the same parts of the embryo as it would produce if left in contact with its fellows that were destroyed. Chabry emphasized the significance of the result in its bearing on the general problem of development.

It is all the more surprising, therefore, that such definite results were neglected at the very time when Roux reached nearly similar conclusions by methods less convincing, because the developing half of the frog's egg remained in contact with the injured and living half. The failure of Chabry's work to make the same impression on his contemporaries, as did that of Roux, may be due, in part at least, to the fact that Chabry's analysis was not carried beyond the point of establishing the mosaic character of the cleavage. He speculated less as to the wider bearing of his evidence. Chabry discussed his results, in fact, more in their relation to teratology than as furnishing important evidence relating to development in general. Furthermore, it was not at first quite clear from Chabry's account, and from his figures, just how far the embryos obtained were really partial structures. For instance he did not show, as Conklin's later work has shown, that the muscles and the mesenchyme of the half-embryo are strictly unilateral in position, although, he did show that the atrium and the adhesive organs were such half-structures. In regard to the origin of the otolith and eye, Chabry's account was very explicit in tracing the absence of these organs to the injury of particular cells that had been removed; but the closure of the archenteron, the total covering of ectoderm, and the form of the tail in the half-larva, might possibly have been interpreted as evidence of whole development, and this, no doubt, was one of the reasons

that led Driesch at first to interpret Chabry's results in conformity with his own, viz., as showing that isolated blastomeres of the Ascidian's eggs produce whole embryos of half-size. How far such an interpretation of particular parts will bear out these conclusions in the light of more recent results will be discussed later.

Not less interesting is Chabry's description of cases in which one of the early blastomeres died, but remained intact and in contact with the living blastomere. The results, as he points out, are strictly comparable to those obtained by his more refined method of artificially destroying particular cells. In fact, it is from observations of just such cases, when death was brought about deliberately by rough treatment of the segmenting egg, that Conklin determined the fate of "isolated" blastomeres of the ascidian's egg. The full significance of Chabry's results was made evident by the much more complete and detailed study of the development of "isolated" blastomeres carried out later by Conklin.

Driesch ('95) studied the development of isolated blastomeres of another ascidian, Phallusia. He described the isolated blastomeres as segmenting more or less as wholes. He stated that they gastrulate as wholes and produce whole embryos. Crampton ('97) found that the isolated blastomeres of Molgula segment as parts, but gastrulate as wholes and give rise to whole embryos. It is true, as Chabry had shown, that the embryo from the ½ blastomere resembles a whole embryo of half-size in so far as it has a rounded body and tail, a closed tubular neural plate and round notochord; but nevertheless, as Conklin has shown later, it also lacks the distinctive organs of the missing side. The closure of such tubular organs as the gut and nerve tube might, it is true, be interpreted as a slight change towards wholeness and even the single line of cells in the notochord, in the half-larva, might be interpreted as a whole structure, but otherwise the embryos are half in all organs that are specifically right-and-left structures in the whole embryos, such as the muscles, mesenchyme, papillae and atrial invagination. In the plain meaning of the terms "whole" and "half," there can be no question but that the ½ isolated blastomere gives rise to a half-embryo.

Conklin separated the blastomeres of Styela in the two- or four-cell stages by shaking in a vial half-filled with water, or

by squirting them in and out of a pipette. Under these circumstances one of the blastomeres may be injured, so that it does not develop, while the other continues on its course. The injured cell is not killed, however, but its development is stopped. Its presence does not appear to affect the development of the other part, which rounds up very soon into a nearly spherical form. The only influence shown by the presence of the injured part is seen in the position of the spindle of those cells on the contact

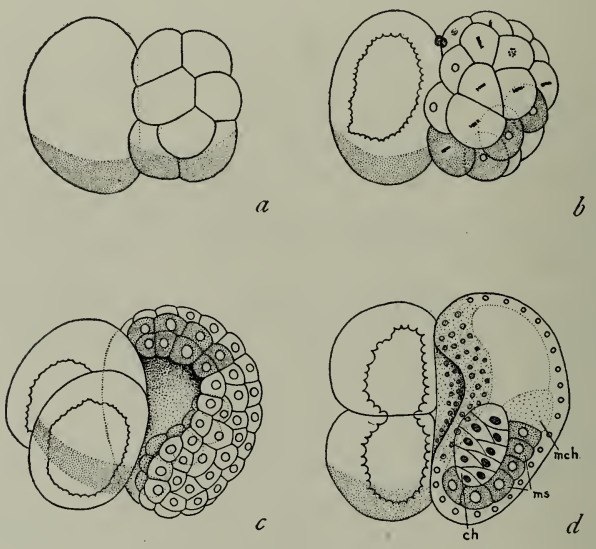


Fig. 129.—Cleavage, gastrulation, and formation of embryo from ½ blastomere of Styela. The injured blastomere is not dead, but its development is delayed. It is still in contact with the more developed half. (After Conklin.)

side that take, at times, a more nearly median or symmetrical position within the more rounded cell than they do in whole eggs.

In the following account, the work of Conklin on Styela is closely followed. The uninjured blastomere cleaves as though still a part of the whole. The cleavage has been followed as late as the 112-cell stage, and even further for certain cells. The isolated blastomere before the next division becomes nearly round. The spindles for its next two cleavages are parallel to the (old) median plane and are not shifted, but in the following division the spindles are moved a little nearer to the old median plane

than in the normal embryo, and consequently, after division, the cells of the median plane come to lie nearer together as though closing over the open side. Minor changes are found also in later cleavages, but the tempo of division, the relative sizes of the cells, etc., are essentially like those in the normal embryo (Fig. 129*a*, *b*). The half-gastrula contains just half as many cells as does the normal gastrula, and the position of the cells is like that of half of the whole embryo of the same stage. For example, the cells of the yellow crescent lie along one side of the blastopore groove (Fig. 129*c*). The neural plate-cells and the chorda-cells form half of the arc that is normally present on the anterior lip of the whole blastopore. The closure of the open side of the gastrula is chiefly accomplished by the overgrowth of the ventral ectoderm cells.

The anterior end of the half-embryo remains large, the posterior end becomes long and tubular to form the tail which contains the notochord and muscle cells (Fig. 129*d*). In the head-end, the neural plate folds in, and one or more pigment spots develop; they may be more numerous even than in the normal embryo, possibly due to certain cells not coming together. The cells of the notochord form a single line of cells, as in the normal embryo, but there are only half as many of them in the half-embryo as in the normal. The muscle cells give rise to three rows of cells along the lateral border of the notochord; and the mesenchyme cells form a group anterior to the muscles. In later stages the muscle cells extend somewhat over to the other side from which they have been lacking. This extension takes place around the end of the notochord and under its ventral side. To this extent, the tail becomes more complete, but there is no increase in size, nor is the missing half replaced. It is obvious that the notochord would interfere with such a regulation, even were it otherwise possible.

If, at the four cell stage, the two posterior blastomeres are killed, the two anterior cells continue to develop as though the other half were present (Fig. 130*a*, *b*). The cleavage is strictly like that of the anterior half of the whole embryo, and the gastrulation is the same, but the late development is modified. There is lacking the mesenchyme, the caudal endoderm, and muscle cells, but the neural plate, chorda plate and gastral endoderm are present. The neural plate seems not to fold over or only in part,

while the chorda-cells never elongate to form a notochord. No tail develops.

Conversely, if, at the 4-cell stage, the two anterior blastomeres are injured, the cleavage of the two posterior cells takes place as though the other half were present (Fig. 130*c*, *d*). The caudal endoderm and the arc of mesenchyme are invaginated, and the muscle cells are overgrown by the ectoderm. "Owing to the absence of the anterior lip of the blastopore and of the notochord

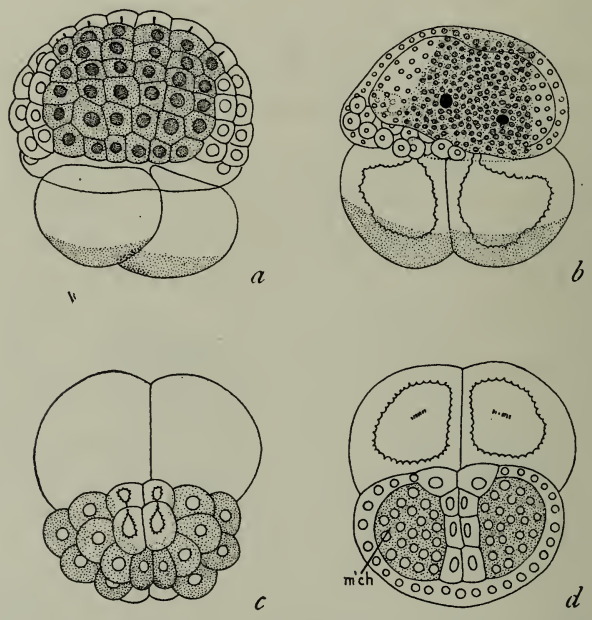


Fig. 130.—*a* and *b*, development of the two anterior blastomeres of Styela; *c* and *d*, development of the two posterior blastomeres. (After Conklin.)

and neural plate, the later stages in the development of these posterior half-embryos is much altered. In the first place the blastopore groove and the muscle cells are not pushed to the posterior end of the embryo. Then the muscle cells on each side of the blastopore are not kept apart by the notochord, but come into contact forming a continuous layer of muscle cells across the dorsal side. The blastopore groove, therefore, disappears by the fusing of the lateral lips of the groove and the ectoderm cells grow over the whole dorsal surface; the only trace of the blasto-

pore groove that is left is the slight notch in the anterior border of the embryo. The ectoderm never entirely encloses the posterior half-embryo on the side next to the injured cells, and the endoderm here comes to the surface. No trace of notochord, neural plate or sense organs ever appears in these posterior half-embryos, and what is more remarkable, a tail never forms, but the embryo always remains rounded in form" (Conklin). In general, the two posterior blastomeres give rise to the same parts that they produce in the normal embryo (Fig. 130*c*, *d*).

The development of the isolated blastomeres of the 4-cell stage is also strictly partial, and gives rise to parts of embryos that are often abnormal (Fig. 131*a*, *b*). The kind of structures or organs that they give rise to depends on which of the four

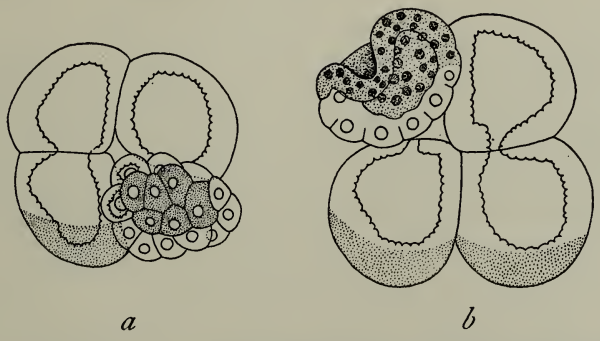


FIG. 131.—Development of a ¼ blastomere, in contact with the other three that have been delayed (injured) in their development. (After Conklin.)

blastomeres develops. The development corresponds to the kind of development that the same blastomere follows when it is a part of the whole. Similar results are obtained for an isolated one-eighth, or one-sixteenth blastomere, although the embryos are quite abnormal in form in these cases, but no more so than expected from the incompleteness of the part in question.

Conklin has laid emphasis on the fact that special regions of the eggs that are often visibly different from each other go to definite parts of the embryo. He believes these regions contain special protoplasmic materials that have a determinative influence in that they give the specific character to the cells that contain them. In a sense this must be true since the isolation experiments show that when the blastomeres are separated they continue to

develop as they do when together, but whether the *visible* specific materials are the determining agents is not apparent. In the first place a careful scrutiny of the facts shows that, aside from the yellow pigment in the crescent, the regional differences are by no means so sharply defined as is sometimes implied. In general, barring the pigment, there are really only two obviously different visible substances, viz., the "protoplasm" and the yolk. The relative amounts of each in the different regions, and to some extent their relative positions is what gives the visible differences. Now in other eggs it has been shown by centrifuging that the yolk has no specific influence on the fate of the cells, and the evidence is so explicit and far-reaching that, I think, we need not hesitate to apply it also to the ascidian egg. The same statement may be made in regard to pigment, in general, and probably, therefore, to the yellow pigment of the Styela egg. On the other hand, it should not be forgotten that the movements of the protoplasm that are a conspicuous feature of the ascidian egg, and which appear to be significant for the future development, are made visible by two of the inclusions in the protoplasm, viz., the yolk and the yellow pigment. It may be claimed, therefore, that even if we reject the hypothesis that these substances are themselves determinative agents they mark out the different protoplasmic regions that are themselves determinative. If this is true, it may be undesirable to lay too much emphasis on the value of these two materials further than as indices of topographical value.

The Development of Isolated Blastomeres of Ctenophores

The eggs of the ctenophore, Beroë ovata, have been found excellent for experimental work, since they are large and clear, and, when cut, do not disintegrate. The following description applies to the eggs of this species alone. The early observations of Chun ('80) on Eucharis were followed by the experiments of Driesch and Morgan ('95) on Beroë. More detailed experiments on the latter were later carried out by Fischel ('97, '98, '03) and by Yatsu ('10, '12).

The egg when laid is covered by a thin, gelatinous envelope that becomes a thick jelly layer after fertilization (Yatsu). Over its outer surface, the egg has a very thin, semi-fluid layer free from granules and difficult to detect. Beneath this is a rather

thick layer of ectoplasm that has a fine alveolar structure. This layer emits a greenish phosphorescent light if the egg is stimulated electrically. The interior of the egg is composed of a coarse alveolar material. The interior of the alveoles is more fluid than

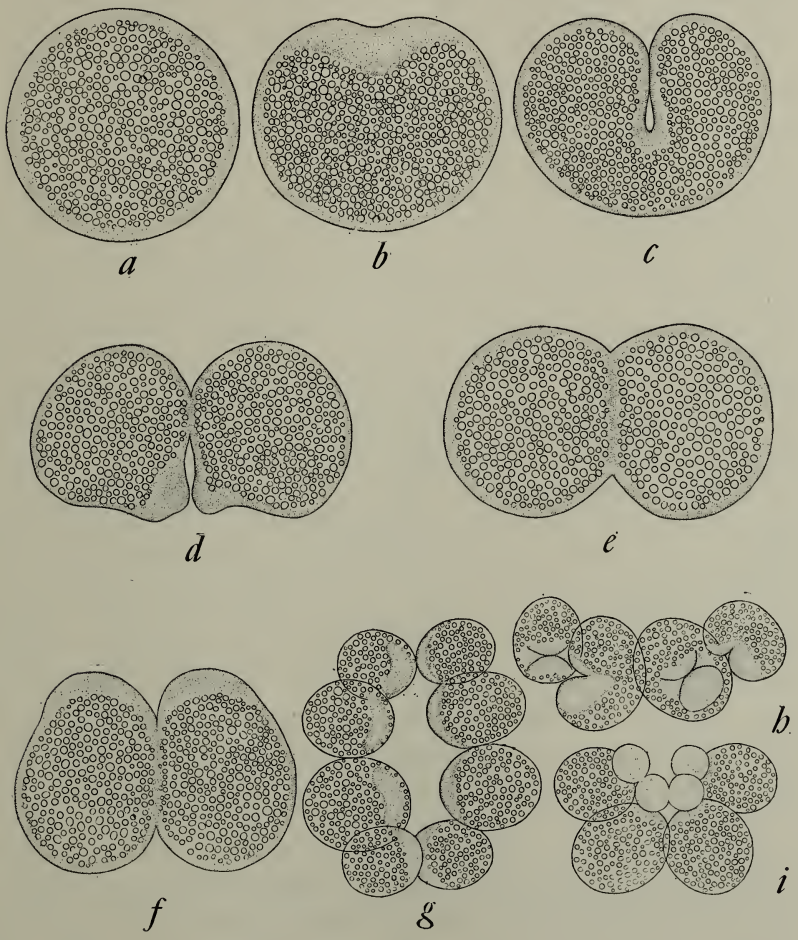


FIG. 132.—Cleavage of the egg of the ctenophore, Beroë, in dark field. The outer layer of the ectosarc sends out emerald green rays in the living eggs. (After Spek.)

ordinary yolk, and is dissolved by most preserving fluids. The nucleus lies in the ectoplasm beneath the pole, and is invisible in the living egg. Two polar bodies are given off. The sperm may enter at any point.

Prior to the first cleavage, an accumulation of ectoplasm appears at the pole. Here, a shallow depression develops, that deepens into a furrow that extends around the egg (Fig. 132*b*). As the pole depression deepens a "cleavage head" of thickened ectoplasm forms at its bottom. A fine display of spinning activities from the walls into the furrow becomes apparent (Yatsu). The alveoles of the ectoplasm become arranged radially. The alveoles of the endoplasm around the walls of the cleavage furrow appear as though drawn out along the furrow as it deepens; while those underneath the "cleavage-head" are flattened. Their arrangement gives the impression that the cleavage-head pushes downward, rather than that it is being pulled down by contraction of rays. With the advance of the cleavage-head, the ectoplasm thickens at the antipole, and later the cleavage-head fuses with this thickening. The alveoles of the endoplasm retreat from this region, and an ectoplasmic connection is, for a moment, left between the two cells. This breaks and the first division is finished.

After the completion of the first cleavage, an ectoplasmic accumulation appears at the polar end of each blastomere. Here the second cleavage furrows start. They progress in the same way as did the first furrow, producing four equal cells (Fig. 133*b*). The third cleavage also begins at the polar end. It divides each cell into two somewhat unequal halves lying nearly in one plane (Fig. 132*g*, 133*c*), giving a bilateral pattern. The micromeres are formed at the fourth cleavage (Fig. 132). Each cell divides unequally (Fig. 133*d*). The four inner micromeres are a little larger than the four outer ones. Each is composed entirely of ectoplasm. Subsequently each of the middle micromeres gives off one more micromere, while the outer micromeres give off two more. After the micromeres are budded off each macromere divides into two equal cells producing sixteen endoderm cells.

The later stages of development include the turning in of the large endoderm cells (or rather their overgrowth by the ectoderm), the formation of some mesenchyme tissue, and an ectodermal oesophagus. An apical ciliated plate, with sense organs, is formed at the anterior end, and, radiating from this, are the eight rows of peculiar combs or "paddles" composed of fused cilia.

The earliest observations on the "half-development" of the egg of one of the ctenophores was made by Chun ('92). He

found in the surface "skimming" two embryos of Eucharis multicornis in the same egg-coat, each of which had only four rows of paddles (combs), and one tentacle instead of eight rows and two tentacles. He inferred that these had come from one egg whose first two blastomeres had been separated. He confirmed this conclusion by shaking two-cell stages, and obtained half-embryos from each blastomere.

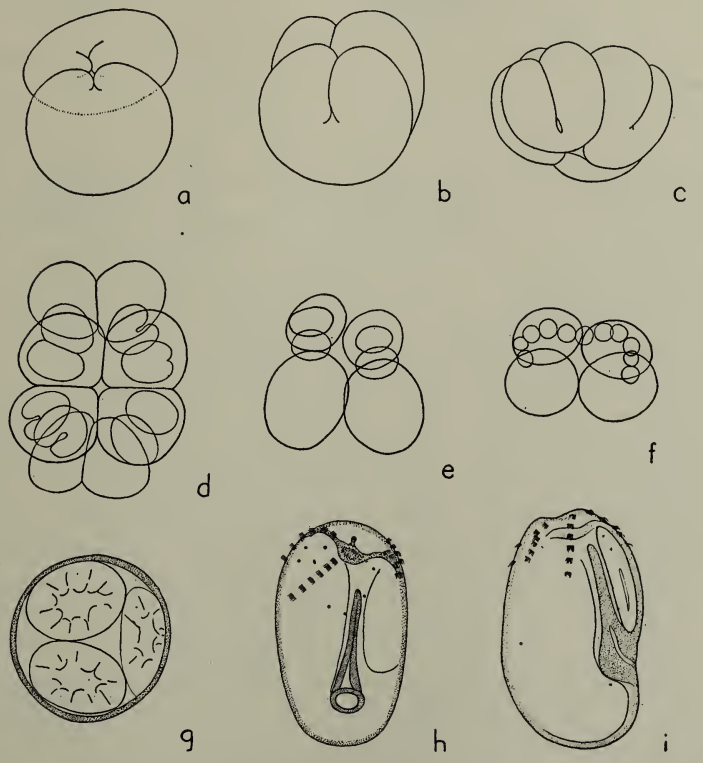


FIG. 133.—*a–d*, cleavage of whole egg of Beroë; *e* and *f*, cleavage of isolated ½ blastomere; *g*, optical section through ½ embryo, showing two complete and one smaller gut-pouch; *h* and *i*, two views of ½ embryo. (After Driesch and Morgan.)

A more systematic study of isolated blastomeres of another ctenophore, Beroë ovata, was carried out by Driesch and Morgan ('95) and later more fully still by Fischel and Yatsu. Driesch and Morgan found that the cleavage of the isolated blastomeres is strictly partial (Fig. 133*e*, *f*). The embryos are halves, as Chun had described for Eucharis, but in addition to the two

endodermal pouches there is a smaller third pouch generally present on the defective side making the embryo a little more than a half-structure (Fig. 133*g*, *h*, *i*). The invaginated oesophagus pushes in somewhat obliquely between these pouches.

The ¼ blastomere also segments as a part and produces a partial larva. It has only two rows of paddles, but two endodermal pouches; the pouch nearest the original center (primary axis) is, however, smaller. The invaginated oesophagus is displaced somewhat to one side, and passes between the two pouches, each still somewhat on one side. Fischel found that ⅛ blastomeres produce larvae with only one row of paddles; 8/16 blastomeres (composed of four macromeres and four micromeres) produce four rows of paddles (like half-larvae). Other combinations of cells isolated at the 16-cell stage give the number of pouches, etc., expected from the preceding results. A group of ¾ blastomeres produces a larva with four pouches. If the micromeres are removed they produce a small spherical mass with eight rows of paddles. Fischel found that if, after cleavage, the micromeres are displaced by pressure, the rows of paddles are irregularly distributed and the apical sense organs may be doubled.

All these results show that there is a close relation between the first cleavages and the localization of the parts of the embryo, but whether the relation arises through distribution of protoplasmic regions of the egg during cleavage, or through other changes taking place at this time is not evident from these experiments. The experimental evidence from egg fragments has, as pointed out in Chapter XV, shown that certain of the differential changes have begun to take place before the cleavage furrow appears.

Numerous isolation experiments with segmented eggs later than the 2- and 4-cell stages have been carried out by Fischel ('97, '98, '03) and by Yatsu ('12). When one of the end-cells of the 8-cell stage (Fig. 134*a*) is isolated, it produces a small embryo with one row of comb plates, one endodermal pouch, a sense organ, but is without a stomodaeum. Two end-cells produce an embryo with two rows of comb plates, one pouch, a sense organ and no stomodaeum (Fig. 134b^1).

Two middle cells of the 8-cell stage sometimes produce an embryo with two rows of combs, a sense organ, but no stomodaeum (Fig. 134d^1). These embryos were quite variable. The results are consistent with Fischel's conclusion that each blastomere of

the 8-cell stage is responsible for one comb-row. That there is some flexibility in this regard is shown by another observation of Yatsu's, where exceptionally and rarely he found that the number

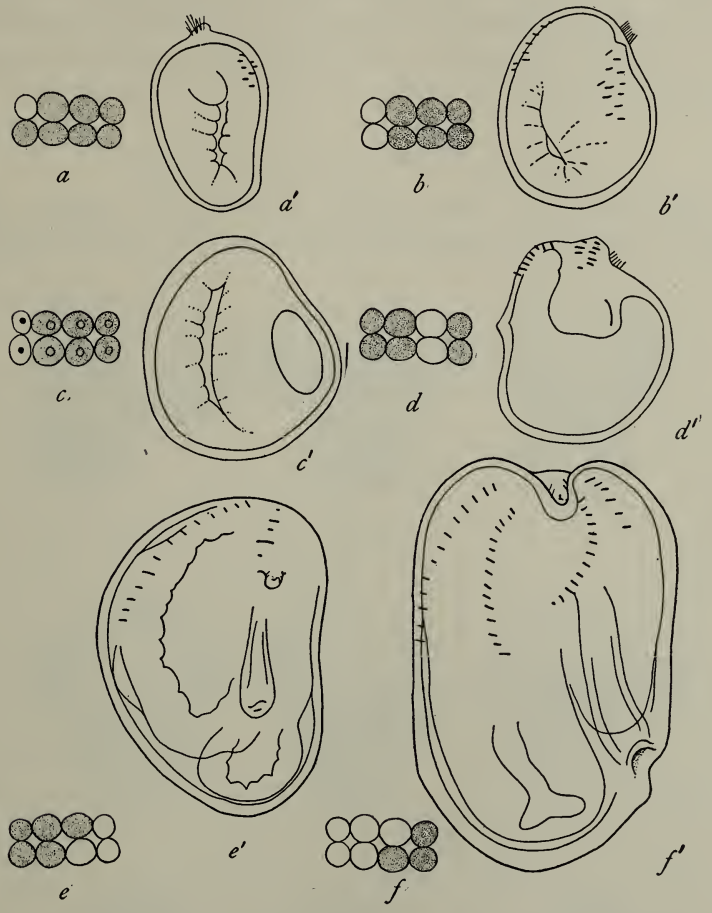


Fig. 134.—Development of isolated blastomeres and groups of blastomeres of the eight-cell stage of Beroë; *a* and *a'*, development of ⅛ blastomere (open circle in *a*); *b* and *b'*, development of two ⅛ blastomeres; *c* and *c'*, same; *d* and *d''*, development of two inner ⅛ blastomeres; *e* and *e'*, development of three ⅛ blastomeres; *f* and *f'*, development of five ⅛ blastomeres. (After Yatsu.)

of comb-rows might be greater (or less) than that of the original blastomeres. For example, two end-cells and one middle cell produced in four cases embryos with four comb-rows, although usually three, and sometimes only two.

The Development of Isolated Blastomeres of Molluscs

The development of isolated blastomeres of four different species of marine gastropods has been studied. They have practically the same cleavage pattern, and the isolated blastomeres develop in the same way. Each type shows, however, certain differences in detail.

Isolated Blastomeres of Ilyanassa

The blastomeres were isolated by Crampton ('96). The eggs in the desired cleavage stage were removed from the capsule,

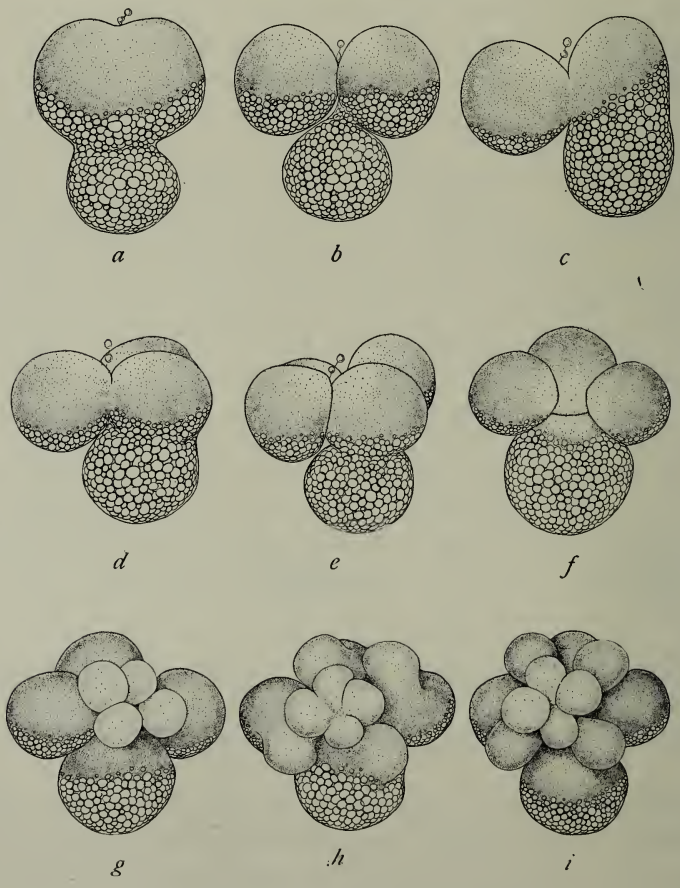


Fig. 135.—Cleavage of egg of Ilyanassa.

and the blastomeres were separated by squirting the water containing the eggs in and out of a pipette. At other times a stream of water that forced the eggs out of their gelatinous capsule sufficed to separate the blastomeres. The eggs are so delicate that many of them go to pieces when treated by either process, but those that pass through the ordeal develop further.

The egg (0.18 mm. in diameter) is opaque, and is without a

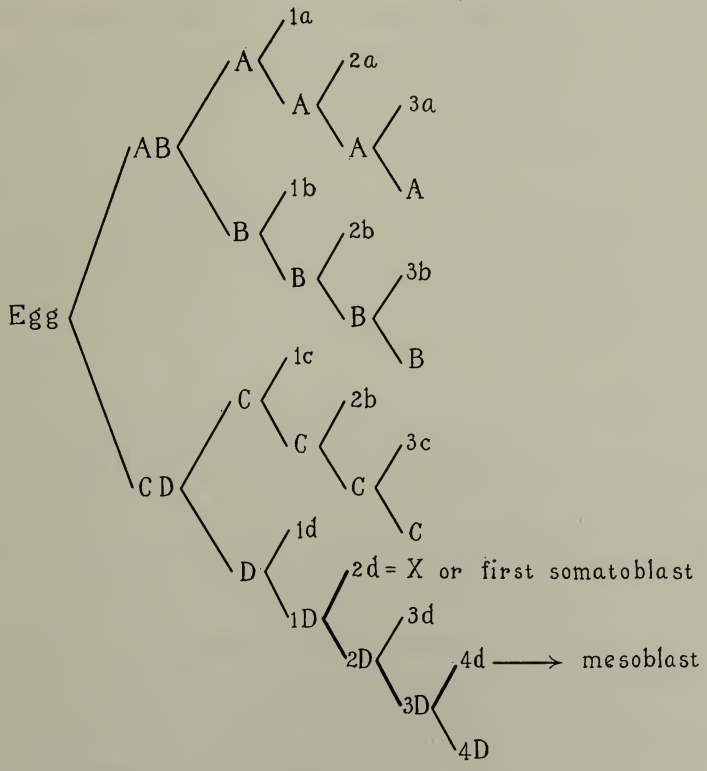


Diagram IV

membrane. Four hours after the second polar-body has been extruded the fertilized egg begins to segment. A swelling at the antipole marks the location of the "yolk-lobe" (Fig. 135*a*). The first cleavage furrow begins to appear soon afterwards at the pole (Fig. 135*a*, *b*). It cuts through to the equator leaving the undivided lobe attached to one blastomere (CD). The second cleavage begins three hours later. The smaller, AB cell, divides

into nearly equal cells; the larger one forms a yolk-lobe and divides unequally (Fig. 135*d-f*). Two hours later four micromeres are given off around the pole by a dexiotropic division (Fig. 135*g*. The yolk-lobe may disappear in the D-cell at the same time. Later a second quartet of micromeres is formed by a leiotropic division (Fig. 135*h, i*), and at about the same time the first quartet divides leiotropically into unequal cells. A third quartet is formed by a dexiotropic division and the second quartet also divides dexiotropically. At the fourth division of the macromeres, each of the three equal macromeres gives off, leiotropically, an entomere containing yolk, and the fourth, larger

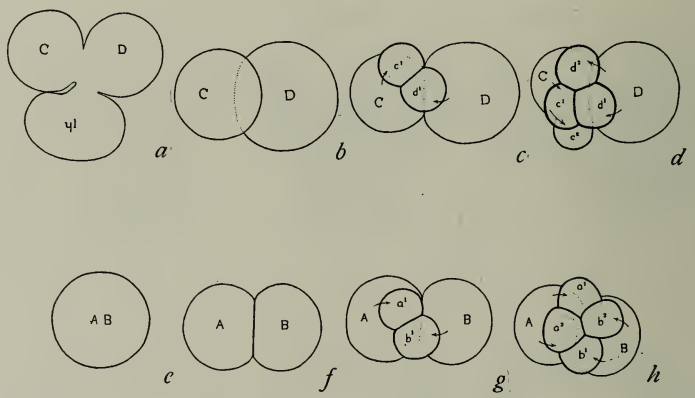


Fig. 136.—*a–d*, cleavage of isolated ½ (CD) blastomere of Ilyanassa; *e–h*, cleavage of isolated ½ (AB) blastomere of same. (After Crampton.)

macromere divides leiotropically also giving off a cell, 4d, the mesoblast pole-cell that does not contain yolk. Soon after its origin the cell, 4d, is divided equally by a vertical plane, establishing the bilateral symmetry of the embryo. The cleavage of Ilyanassa is essentially the same as that of other molluscs, such as Dentalium (see Diagram IV), and Crepidula, and the lamellibranch, Unio, and resembles in all essential points that of the annelid Nereis.

When the first two blastomeres are shaken apart each develops nearly as it would have done in the whole embryo. The smaller blastomere (AB) becomes perfectly spherical, and cleaves as a part (Fig. 136*e-h*). Its first division is into equal parts (A and B). Each of these macromeres produces by a dexiotropic

division a micromere. After the usual pause, each macromere produces leiotropically another micromere, one of them slightly altered in position. In later divisions no evidence of the 4d cell is present in such embryos. The embryo is a half-embryo with a "partial circle of cilia." It soon dies.

The larger of the first two blastomeres (CD) produces a yolk-lobe, when about to divide, and cleaves into unequal parts (Fig. 136*a-d*). At the next division two micromeres are formed, etc. In one case at least, a 4d cell was found which was identified by its position, its clear protoplasm, and its early subsequent division. The partial embryo that arises soon dies.

When the yolk-lobe is cut off from the larger of the first two isolated blastomeres (CD), the remaining cell divides, as does its partner, without the lobe.

The isolated ¼ blastomeres (A, B, C or D) round up and each gives off a micromere. Later, another micromere is formed, and soon afterwards, the first micromere divides equally. After the usual rest a third micromere is given off and the second micromere divides equally. A few more divisions take place before the embryos die.

The isolated ⅛ macromeres do not divide. The isolated micromeres may divide once or twice, but soon die.

The yolk-lobe can be removed from the egg at the two-cell stage. The cells then continue to divide as they do in the whole egg, but when the fourth division takes place, the four cells, 4a, 4b, 4c, 4d, are all alike in size, that is, the 4d cell cannot be distinguished from the others, since the yolk-lobe material is absent from it. No mesoderm develops; the embryo is made up of ectoderm and endoderm. The result appears to mean that although the D-cell, without the yolk-lobe material, produces the 4d cell, the absence of the material of the yolk-lobe in it is connected with the absence of mesoderm.

Crampton found that when normal unsegmented eggs were taken out of the capsule and kept cool over night, and then brought back to normal temperature, some of them divided normally, but others had the yolk-lobe much reduced. The eggs might even divide without forming a yolk-lobe, but such eggs divided abnormally. The isolated blastomeres from these eggs divided irregularly. In some cases, Crampton thought the division simulated the cleavage of whole eggs, but this may seem doubt-

ful, for, owing to the great irregularity, it is difficult to determine whether such a type may be only an irregularity rather than a whole cleavage.

Isolated Blastomeres of Dentalium

The cleavage of the egg of Dentalium (Figs. 90, 92), has been described in the chapter on egg-fragments.

The embryos that develop from ½ and ¼ isolated blastomeres have been studied by Wilson ('04). The cleavage of isolated blastomeres of Dentalium takes place as though the blastomere were a part of the whole, i.e., the cleavage is partial (Figs. 137, 138). When the AB and CD blastomeres are isolated they continue to cleave and ultimately close in, gastrulate and produce swimming larvae, but they show the following constant differences.

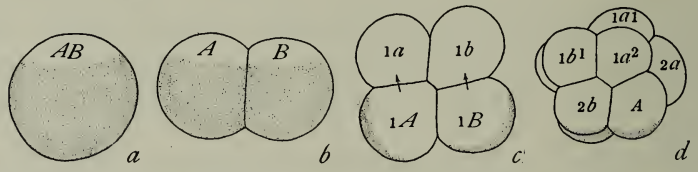


Fig. 137.—*a–d*, cleavage of isolated ½ (AB) blastomere of Dentalium. (After Wilson.)

The smaller larva (from the AB blastomere) lacks the post-trochal region and the apical organ (Fig. 139*d*), as does a whole egg from which the lobe has been removed. Clearly the latter result is in some way due to the absence of the lobe. The larva is partial in these respects. In the larger larva (CD), both the post-trochal region and the apical organ are present (Fig. 139*c*), and as large as in the whole normal larva; therefore proportionately too large for the half-larva. Superficially, at least, the CD embryo is not partial, but as yet we do not know whether the mesoderm from the 4d cell forms a symmetrical or an asymmetrical structure inside of this embryo. Outwardly, at least, the CD embryo approaches a whole larva of half-size, but if we apply to this case the same criteria that have been insisted upon in the case of the half ascidian embryo, we should be obliged to confess that we do not know whether the half Dentalium, CD-trochophore is a half or a whole structure. Much less could it be said that the AB-trochophore is a whole embryo, if it lacks

all the mesoderm normally derived from the 4d cell. The results that Penners has obtained after isolation of the D cell of the annelid, Tubifex (to be described later), make it probable that the CD embryo of Dentalium has a typical bilateral mesoderm.

When the first four blastomeres are isolated, three of them lack the two organs in question, while the fourth blastomere (D), which is the largest, develops them. The two regions are, however, proportionately "very much too large."

The ¼ blastomeres when isolated divide as though still part of a whole. The A, B and C quadrants divide slightly unequally without a polar lobe (Fig. 140*a*); the D quadrant forms a polar

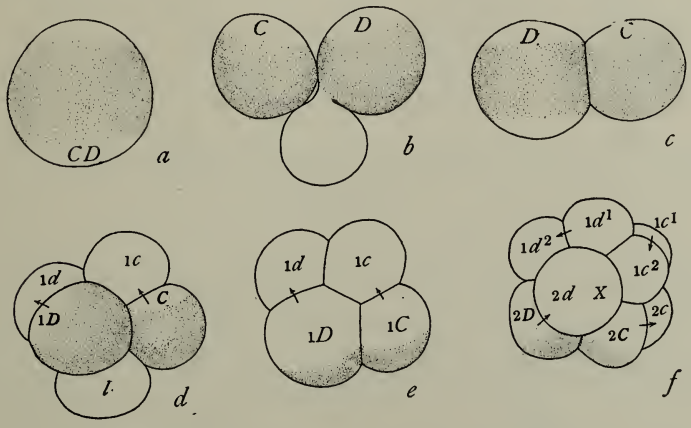


Fig. 138.—*a–f*, cleavage of isolated ½ (CD) blastomere of Dentalium. (After Wilson.

lobe and divides very unequally (Fig. 140*b*, *c*), the smaller cell (1d) being pure white, the larger showing both the upper and lower polar area.

Wilson has also isolated the blastomeres of the later cleavage stages, and has studied their subsequent development. The first micromeres when isolated continue to divide, producing a cluster of small cells, but the micromere (1d) from the D-quadrant differs from all the others in that it produces an apical tuft of cilia (apical organ). On the other hand in the mollusc Patella that has no yolk-lobe, each of the first micromeres, when isolated, gives rise to a closed ectodermal structure having an apical organ at the anterior end, and a group of active trochoblasts at the posterior end.

During the "trefoil" stages of the first cleavage, Wilson found that the yolk-lobe may be easily removed by cutting it off with a fine scalpel. The operation leaves the CD cell about the same size as the AB cell. The egg continues to segment quite symmetrically, and the cleavage pattern represents that of such forms

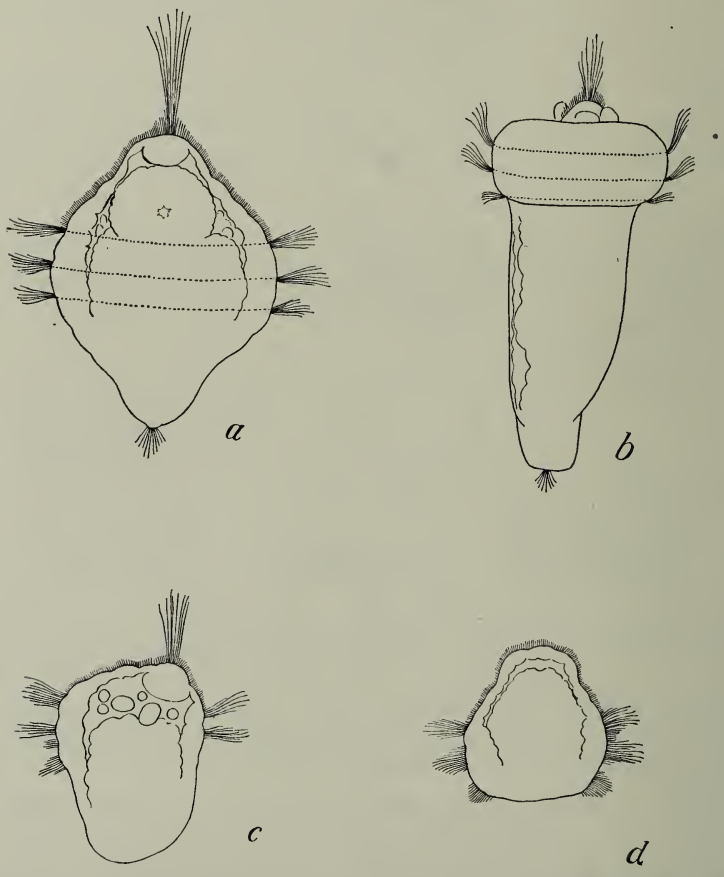


FIG. 139.—*a*, *b*, normal, swimming embryos of Dentalium; *c*, *d*, embryos from ½ blastomeres of Dentalium. (After Wilson.)

as Patella or Lymnaea. No further trace of the yolk-lobe is to be seen in the second or third cleavages of the lobeless larvae. There is no longer a lower, white area as in the normal embryo, and sometimes there is a large opening into the cleavage cavity. The lobeless embryos gastrulate and produce spirally swimming

embryos that differ, however, from the normal in two respects. The post-trochal region is absent, and there is no trace of an apical organ. The absence of the trochal region accords with expectation, for, as Wilson has shown, this region is derived from the two somatoblasts that come from the yolk-lobe region. The absence of the apical organ is more difficult to interpret, since it arises from cells on the opposite end of the egg.

If part only of the yolk-lobe is cut off, then, during the second and third cleavages, the yolk-lobe is correspondingly diminished in size. There is also a reduction in the post-trochal region, and

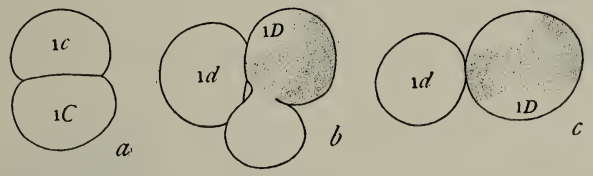


FIG. 140.—*a*, first cleavage of an isolated ¼ blastomere (C) of Patella into 1C and 1c; *b*, *c*, first cleavage of an isolated blastomere (D) of Patella into 1D and 1d. (After Wilson.)

these larvae sometimes possess, sometimes lack, the apical organ. "Removal of the second yolk-lobe produces a larva without post-trochal region but with an apical organ. The lobeless larvae undergo no metamorphosis, form no foot, shell-gland or shell, no mantle folds, no pedal ganglia, apparently no mouth, and probably no coelomesoblast-bands."

Isolated Blastomeres of Patella

The egg of the mollusc, Patella, cleaves in essentially the same way as that of Dentalium, except that there is no yolk-lobe, and, in consequence, the first two blastomeres are equal. The three quartets of ectomeres are successively formed by alternating dexiotropic and leiotropic divisions.

Wilson ('04) has isolated the blastomeres. At the end of the first division, the two cells were cut apart with a scalpel and placed in pure sea water. The cleavage was partial. Many of the embryos developed abnormally, but it was found that both halves developed an apical organ. While it was not shown that this also happens in the case of the isolated ¼ blastomeres there can be little doubt that they have the capacity to do so, because

it was found that any micromere of the first quartet may develop an apical organ. "The basis of the apical organ in Patella must, therefore, be symmetrically divided by the first two cleavages while it remains undivided in Dentalium, remaining as a whole in the D-quadrant" (Wilson).

By the use of the Ca-free water Wilson was able to isolate cells from various parts of the segmented egg. These cells continued to divide as though still a part of a whole, and produced a group of differentiated cells of the kind that would have been produced had they remained in contact with their fellows. For example, "isolated $\frac{1}{8}$ macromeres produced closed embryos that gastrulate and bear, at one end, one or two secondary trochoblasts, and at some other point a small group of feebly ciliated

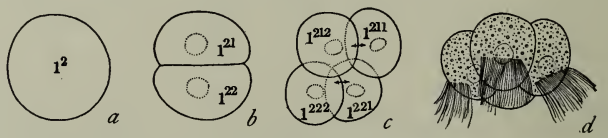


Fig. 141.—*a–d*, cleavage and differentiation of one of the blastomeres of the first quartet 1^2 of Patella. (After Wilson.)

cells like those of the normal larvae. Isolated $\frac{1}{16}$ macromeres produce closed embryos, that gastrulate, bear no trochoblasts, but have feebly ciliated cells" as in the last case. These and similar results from other isolated cells show incontestably that, at a very early stage, the fate of the cells is determined, and that they go through the same number of divisions and produce the same kinds of cells that they produce when they remain in contact with their fellows. Thus, when one of the primary trochoblast cells (1^2) is isolated (Fig. 141*a*), its products form four typical trochoblast-cells (Fig. 141*d*).

Isolated Blastomeres of Crepidula

Although the blastomeres of the mollusc Crepidula are closely adherent to each other, Conklin ('02) succeeded in separating the first two, and even the first four in the late phases of the first and second divisions by means of pressure, or by shaking, or by placing them in dilute sea water.

The $\frac{1}{2}$ isolated blastomere first divides equally in a slightly leiotropic direction (Fig. 142*a*). At the next division each cell

gives rise to a micromere of normal size by a dexiotropic cleavage (Fig. 142*b*, *c*, *d*); then to a second micromere by a leiotropic cleavage (Fig. 142*e*), and to a third by a dexiotropic cleavage, (Fig. 142*f*) exactly as in normal cleavage.

If one of the first four blastomeres is isolated it gives off a micromere by a dexiotropic cleavage, a second by a leiotropic, and a third by a dexiotropic cleavage.

The later subdivisions of the micromeres take place exactly

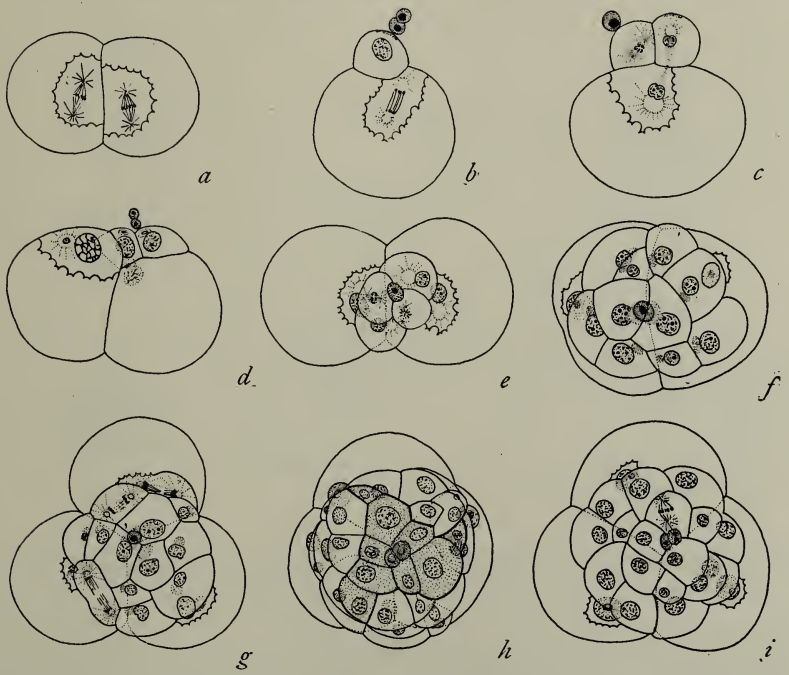


FIG. 142.—*a–i*, cleavage of isolated ½ blastomere of Crepidula. (After Conklin.

as in the normal egg in each quadrant. Finally, in these partial embryos, the 4D macromere, if present, gives rise to the mesentoblast, 4d, which then divides into right and left halves M1 and M2 to form the mesoblast. But if the D-macromere is not present in the isolated fragment no mesentoblast is formed. The results show that in every important detail the isolated blastomeres cleave as do isolated blastomeres of other molluscs.

Isolated Blastomeres of Tubifex

The eggs of the annelid, Tubifex, are inclosed in a common capsule. The cleavage (Fig. 143), is closely similar to that of other annelids, more especially to that of the leech. The chart (Fig. 144), gives the cell lineage. The egg (0.3 to 0.5 mm. in diameter) is filled with yolk. As soon as the polar bodies are given off, a thin plasma layer covers the surface of the egg. The egg flattens at both poles. Amoeboid movements next take place,

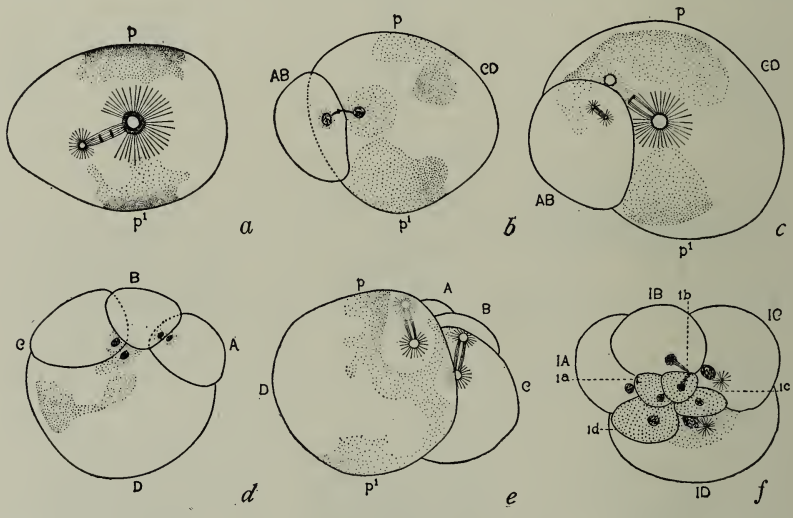


Fig. 143.—*a–e*, sections of cleavage stages of the egg of Tubifex; *f*, reconstruction o eight-cell stage of same. (After Penners.)

but later the egg assumes an oval shape. Extensive shifting of the interior is going on during this time. Two protoplasmic caps appear, one at, or near the pole, the other at the antipole (Fig. 143*a*). The polar field has the form of a ring; the antipolar that of an irregular disc. The polar bodies often shift their position, so that it is not possible to state whether or not the original pole of the egg, where they were eliminated, corresponds with the center of the polar ring, or whether the ring lies to one side of the pole. There is some insufficient evidence that the ring may be slightly excentric to the original pole. The first cleavage is very unequal (Fig. 143*b*, *c*) into AB and CD. The plane of division cuts far to one side of the polar field, but lies nearer

to the antipolar field. Both pole-plasms are left in the large cell (CD), which is 4 or 5 times as large as the smaller cell (AB). Preceding this division the spindle takes a very excentric position, and one of its poles becomes much larger than the other (Fig. 143*a*). During the division the two pole-plasms sink deeper into the egg, and come to lie within the larger cell. Occasionally a little of one or both of these plasms may get pinched off into the smaller cell.

The cell CD next divides into C and D; the D-cell being larger than the C-cell (Fig. 143*d*), contains all or nearly all of the pole-plasm materials. The cell AB then divides nearly equally into

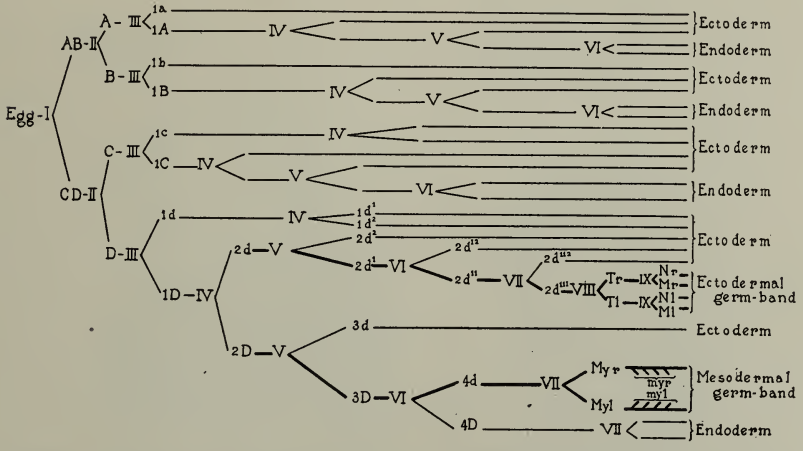


Fig. 144.—Chart of the cell-lineage of Tubifex. The heavy lines in the lower part of the chart give the history of the ecto- and mesodermal germ-bands. (After Penners.)

A and B. A and C lie higher than B and D, the former, with pointed ends, touching each other at the pole, the latter (B and D) meeting at the antipole in a cross-furrow.

At the next division (Fig. 143*f*) four micromeres (1a, 1b, 1c, 1d) are given off at the polar ends of the four cells in a dexiotropic spiral; the formation of 1d and 1c preceding the formation of 1a and 1b. The future median plane lies in the middle of 1B and 1D.

At the next cleavage 1D divides into two nearly equal parts, 2d and 2D (see Fig. 144 for cell-lineage), the former containing little yolk, most of it remaining in 2D. The cell 2d is the first somatoblast, and gives rise to the ectodermal bands, after further

divisions, which become the nerve-cord, etc. At the same time each of the other large cells (1A, 1B, 1C) gives off a micromere (1a, 1b, 1c).

It is not necessary to follow here the subsequent divisions. They are essentially the same as in the leech, Clepsine. The fate of the 2D-cell alone calls for a further statement. It next divides into 3D and 3d. The 3D divides into 4D and 4d. The cell 4D gives rise to endoderm. The cell 4d divides again (Fig. 144) into Myr and Myl, that give rise to the mesoderm bands (Fig. 144).

By means of ultraviolet light rays ("Tschachotins' method")

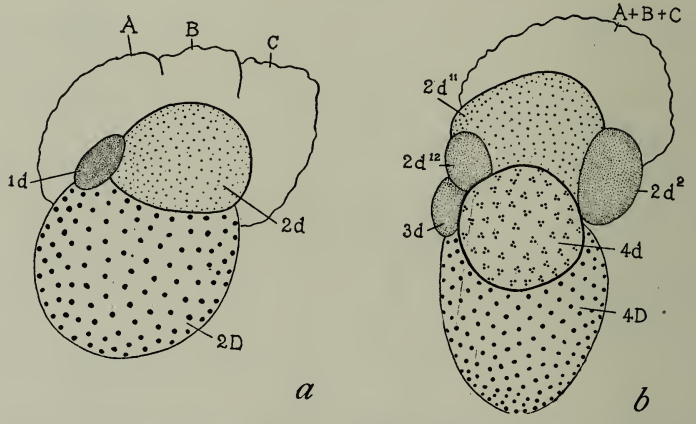


FIG. 145.—Development of an isolated D-blastomere of Tubifex. (After Penners.)

Penners ('24, '26) has injured particular blastomeres of the segmenting eggs of Tubifex, and has followed the development of the uninjured blastomere. After exposure of a cell from one to three minutes to ultraviolet rays the cell is killed and soon becomes disorganized. The other cells are not affected by its presence.

When the three cells A, B, C are killed (at the 4-cell stage, Fig. 145), the remaining cell, D, continues to divide as though still in contact with the other cells (Fig. 145*a*, *b*). It forms its micromeres in normal proportions. The cells 2d and 4d become the two somatoblasts, and form typical germ-bands. A normal whole embryo, somewhat reduced in size, develops. The absence of the products of the three blastomeres A, B, C does not prevent a normal (or approximately normal) embryo from developing.

These three blastomeres, A, B, C, contribute ectoderm and endoderm to the normal embryo. In their absence the ectoderm and endoderm of the smaller embryo come from the D-cell. Presumably it contributes exactly the same ecto- and endoderm cells that it

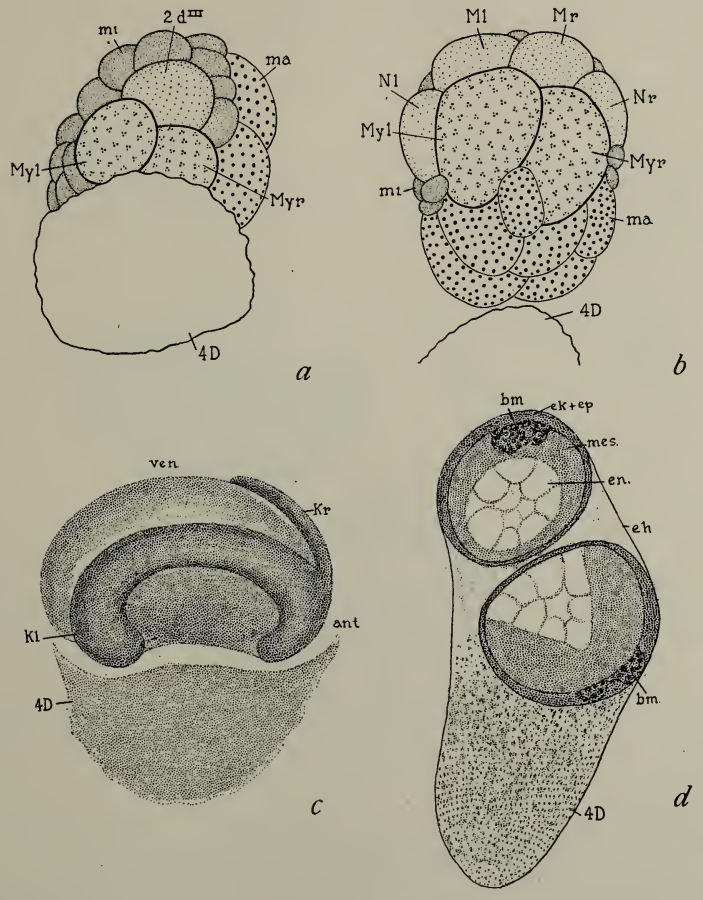


Fig. 146.—Development of isolated group of blastomeres of Tubifex after the 4D cell had been killed. (After Penners.)

contributes under normal conditions to the whole embryo, and the former suffice to cover the smaller D-embryo. The D-cell produces a whole midgut (in the absence of the A, B, C endoderm). Essentially the same result happens when either the A or the B or the C blastomere is eliminated.

When the 4D blastomere is killed with ultraviolet rays, the remaining blastomeres continue to cleave as in the normal egg (Figs. 146*a*, *b*). At the time of operation both 2d and 4d had already been separated in the D-quadrant. Other similar eggs were found in cultures that had been kept cold (10 degrees C.). One of the former gave rise to the embryo shown in (Fig. 146*c*),

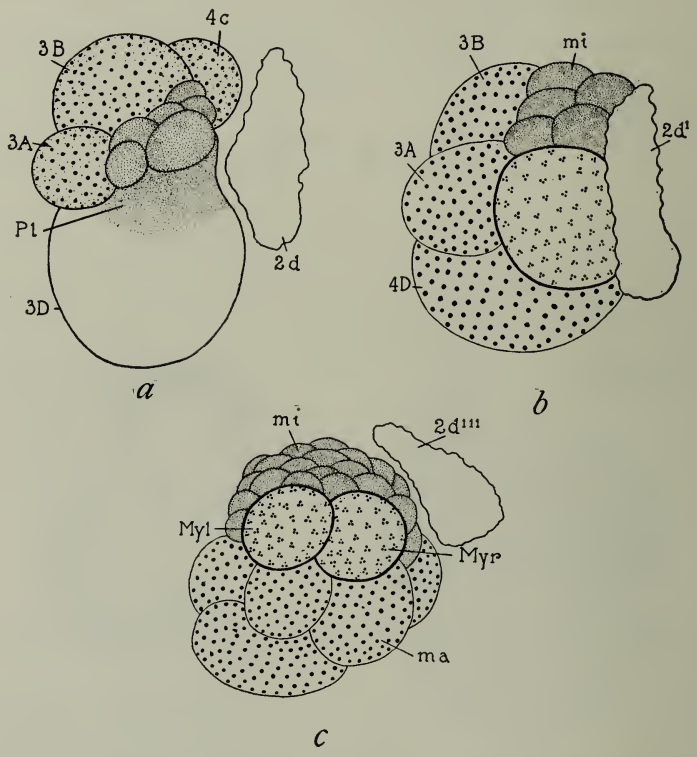


Fig. 147.—Development of isolated group of blastomeres of Tubifex after the 2d blastomere had been killed. (After Penners.)

in which the germ-bands are in process of meeting. Later a normal embryo developed as shown in section (Fig. 146*d*).

The converse experiment consists in killing the CD or the D-blastomere. When CD is killed the AB-cell cleaves as it does normally, and gives rise to ectoderm and endoderm, but to none of the other parts of the embryo.

In still other experiments, the blastomeres, from which the ectodermal bands arise were destroyed, and in others those that

give rise to the mesodermal bands. When, for example, the 2d blastomere was destroyed, the development of the remaining cells went forward as usual (Fig. 147*a*, *b*, *c*, and Fig. 148*a*, *b*). The mesodermal bands, derived from 4d, appeared later and extended around the sides of the embryo. The embryos were somewhat deformed, but all the parts were present except the ectodermal bands, and later their end-products, the central nerve cord, but the mesoderm failed to extend over the dorsal sides of the embryo.

The elimination of the mesodermal germ-bands is brought about by the destruction of the second somatoblast 2D or 4d, or of Myr-Myl (Fig. 149*a*, *b*, *c*). As in the preceding cases, the remain-

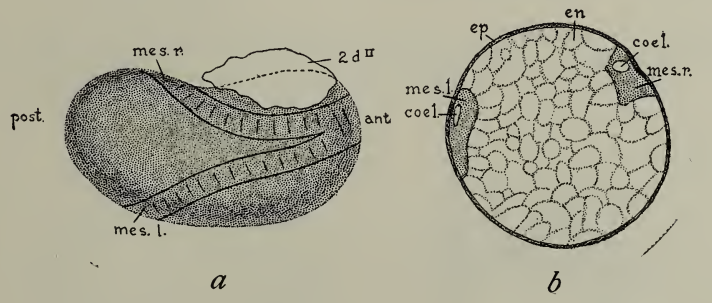


FIG. 148.—*a*, embryo from a group of blastomeres in which the 2d blastomere had been killed (as in Fig. 147). The mesodermal bands are present, but the ectodermal bands are absent; *b*, cross-section of embryo, showing the mesodermal bands and absence of the ectodermal bands.

ing cells continue their normal course of cleavage. The ectodermal germ-bands develop later (Fig. 150*a*). The embryo is normal in form, but sections show (Fig. 150*b*) that the mesoderm is absent.

These results from injuries to those blastomeres that produce the two kinds of germ-bands show that the development of these bands is to a large extent independent, although they take a parallel course in the embryo, but since the later histological differentiation of the bands is somewhat incomplete in the embryos, the inference, that they have a secondary effect on each other, seems probable.

The development of a strictly "half-embryo" can be brought about by destroying the Tr and Myr or Tl and Myl cells (Fig. 151*a*, *b*, *c*). This was accomplished after these cells had appeared,

by killing them on one, or on the other side. The dead cells are soon crowded out by the developing part (Fig. 151*c*). This part proceeds then to form the half germ-bands of one side. Sections show that the further development of such a half proceeds normally. The half-germ-bands develop independently into the half-structures (½ ventral nerve cord, seta-sacs, segmental

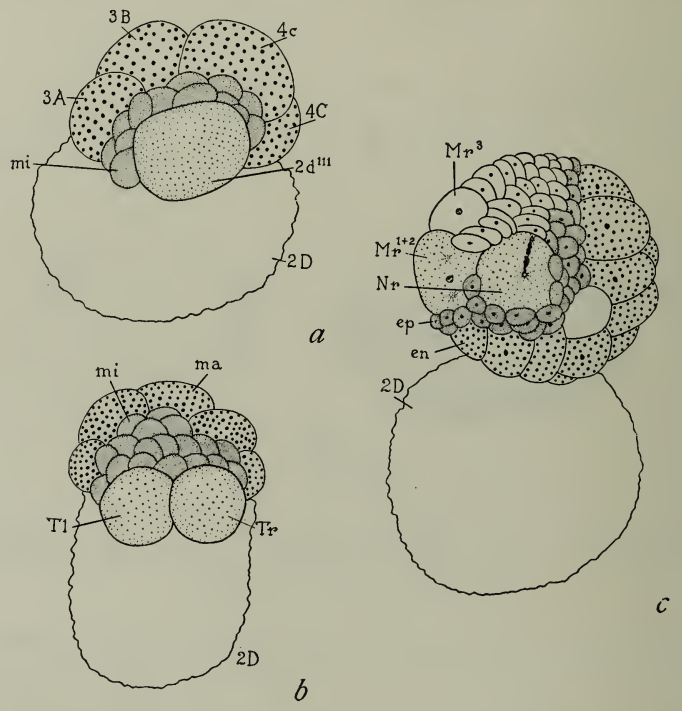


Fig. 149.—Development of group of isolated blastomeres of Tubifex, in which the 2D blastomere has been killed. The ectodermal germ-bands developed later, but the mesodermal germ-bands were absent. (After Penners.)

organs, somatopleure and splanchnopleure of one side) (Figs. 152*a*, *b*).

Throughout the description of these experiments, Penners emphasizes the significance of the materials derived from the two polar fields. He interprets the results in terms of these materials as organ-forming materials. Their presence in the D-cell, and in the two somatoblasts that are derived from the D-cell, determines, as he interprets the results, the subsequent behavior of

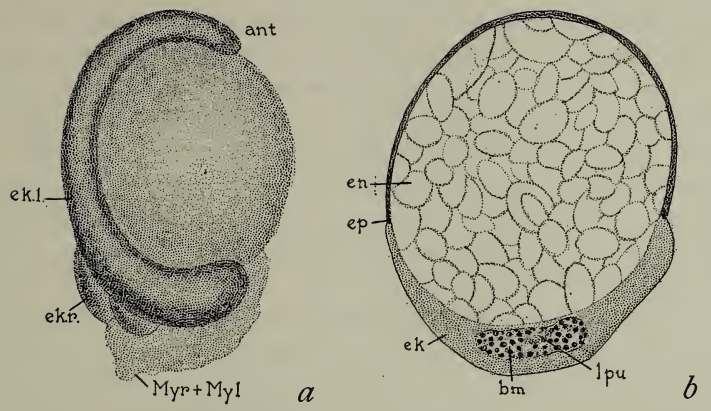


Fig. 150.—*a*, embryo with ectodermal, but no mesodermal germ-bands. It developed from an egg in which the 2D blastomere had been killed, as in Fig. 149; *b*, cross-section of embryo drawn in *a*, without mesodermal bands. (After Penners.)

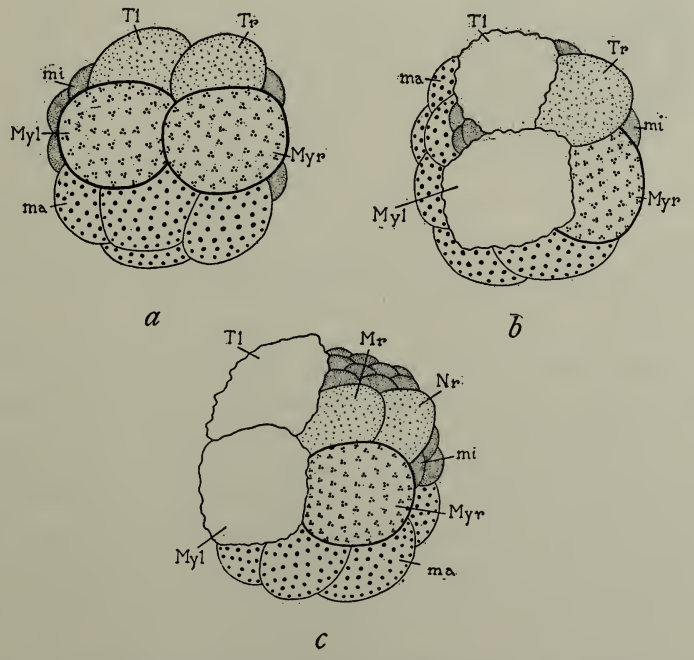


Fig. 151.—Cleavage of isolated ½ blastomere of Tubifex. The other blastomeres, Tl and Myl of one side had been killed. See chart, Fig. 144. (After Penners.)

these cells and their descendants. It is not to be denied that the facts may seem to find a simple interpretation in this way, and that the interpretation finds further support in other observations (to be described later) in which, when the first cleavage divides the egg into two equal blastomeres, each cell, containing half of each of the two polar fields, then produces a whole embryo of half-size. It may also be recalled in this connection that Wilson had earlier observed in Dentalium that when the yolk-lobe that contains the antipolar material is removed, the resulting embryo is defective in certain parts.

A more critical examination of the evidence raises some doubts

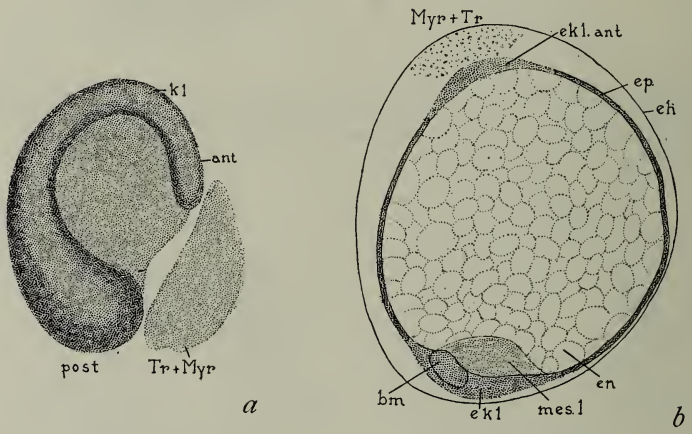


Fig. 152.—*a*, half embryo of Tubifex, and *b*, cross-section of same, from an egg in which Tl and Myl of one side had been killed, as in Fig. 151. (After Penners.)

as to whether the conditions are as simple as Penner's interpretation implies. For example, the mixing of the two materials derived from the poles appears more irregular (in sections of the egg) than one might be led to suppose. It may seem doubtful whether or not these two materials derived from opposite poles of the egg contribute to the same organ after mixing, or whether they are separated at some of the later divisions in such a way that the ectodermal germ-bands are derived from one and the mesodermal germ-bands from the other. There is also evidence that some of this material may occasionally pass into other cells. If this material, or these materials, could be segregated by centrifuging it might appear possible to settle the point at issue, but in the experiments of Schleip with the centrifuge (to be

described later) the pole-plasm materials of the egg of Clepsine cannot be separated, in this way. The irregularities in the cleavage that resulted may themselves sufficiently account for the abnormalities in the later development.

The experimental evidence that Penners has obtained does show, however, quite convincingly that the early development is strictly mosaic as other experiments on eggs of this kind had already made manifest. His results also make clear that the 4D blastomere may produce a whole embryo, but it does not necessarily follow that it has this capacity because it contains the pole-plasms, even though these are a factor in the result. If, for example, it could be shown that its cleavage pattern is directly determined by the presence of the polar materials, this would go a long way in support of his contention, but if, as seems possible, the type of its cleavage is determined by other conditions present in the egg, it might be argued that the cleavage itself is the determining factor rather than the polar materials.

It might, furthermore, be pointed out that even if the pole-plasms are essential for the subsequent differentiation, it may not be the material in them, as such, that is organ-forming, but rather the excess of this kind of material in certain organs—a material everywhere present, but in relatively smaller amounts. It might equally be argued that these visible substances are only indices of a general distribution of the materials that are essential for particular parts. Such a view emphasizes the arrangement, or, if one will, the organization of the materials as the essential feature of this kind of development rather than the importance of the presence of materials as such that are organ-forming. If so, the question involved may seem largely a matter of words or of emphasis, but I cannot but think that there is, nevertheless, an important difference involved that is something more than emphasis. At any rate it seems to me desirable not to close too quickly the issue by accepting the more obvious and perhaps too simple interpretation of the pole-plasms as the actual differentiating substances. While quite fully appreciating the value of these results, the question involved seems to me to be of such fundamental importance for one of the most significant features of the developmental process that the final decision must be left until unequivocal evidence is obtained that will settle the problem.

An important part of Penners' evidence relates, as stated above, to other results with eggs that divide at first into equal parts. The significance of this evidence can best be considered after the facts have been discussed in another chapter.

One point remains. The embryo derived from 1D or 2D is a whole embryo, but it still lacks presumably the cells, or their equivalents, that arise in the normal embryo from the other quadrants. In so far as these are absent, it cannot be said *sensu strictu*, to have regulated its developmental processes to make a whole, but in so far as the cells present readjust themselves to make a complete covering of ectoderm or a tubular digestive tract, there is regulation—but still, perhaps no more than takes place in the normal development for these same kinds of cells.

CHAPTER XVIII

THE DEVELOPMENT OF ONE BLASTOMERE IN CONTACT WITH AN INJURED ONE

The first two blastomeres of the frog's egg are so closely united to each other that they cannot be separated by the methods employed to separate the blastomeres of other animals. If one blastomere is punctured so that its contents flow out, the other blastomere generally collapses. A different method has, therefore, been used in studying the development of single blastomeres. The development of one blastomere is prevented or delayed by injuring it with a hot needle. The method is much less satisfactory for the analytical study of development than the method of isolation. Owing to these drawbacks there has been a great deal of controversy concerning the interpretation of the results. In 1888 Roux injured one of the first two blastomeres of the frog's egg by plunging the point of a heated needle into it. The further development of the injured blastomere was interfered with, while the uninjured sister-blastomere continued to segment. It segmented as though the other blastomere were also segmenting.

The injured blastomere is not dead and remains intact, but in the path of the hot needle its protoplasm is coagulated, and the division of the nucleus is to some extent suppressed. The two blastomeres remain flattened against each other, both retaining their hemispherical form.

The details of the gastrulation were not worked out by Roux, but have been more fully described by later observers. In general it may be said that the dorsal lip of the blastopore appears at the normal level on the uninjured half, and, in conjunction with the lateral lip of its side, closes over the yolk of one hemisphere along the border between the developing and undeveloped half. Later, half of the neural plate appears along one side of the line of contact of the two halves. It rolls over to form half of a

neural tube (Fig. 153*A*). The anterior end of the neural plate, from which the brain develops, appears in most cases to be something more than half, approaching a whole brain of somewhat smaller size. Cross-sections through the middle of the "half embryo" (Fig. 153*D*) show the following structures: there is a

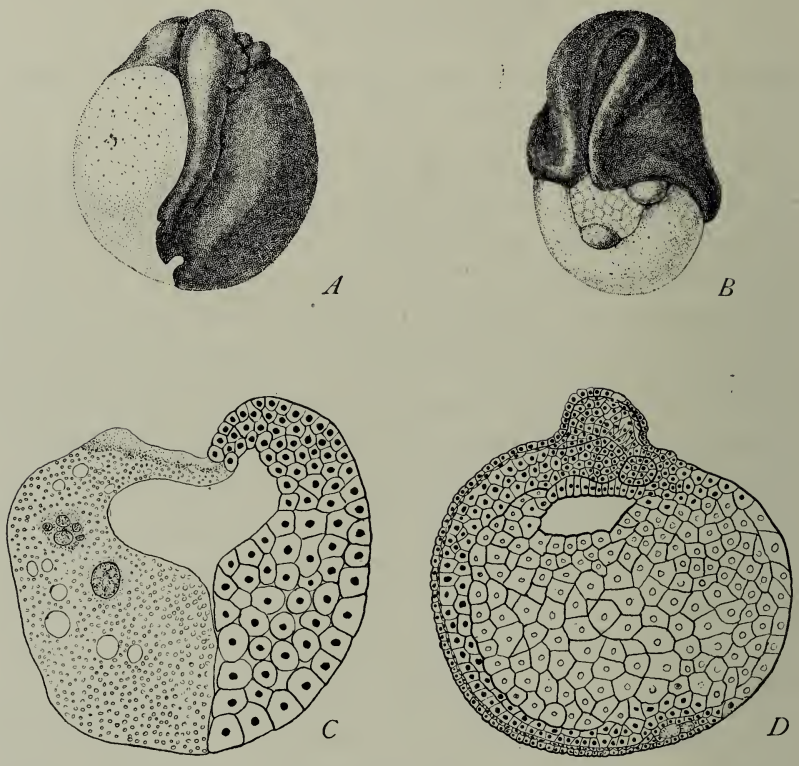


Fig. 153.—*A*, half-embryo of frog, resulting from injury to one of the first two blastomeres; *B*, anterior embryo, so-called, after injury to one of the first two blastomeres of an egg in which the first cleavage had been at right angles to median plane. This embryo may also be interpreted as a whole embryo in which the lateral lips of the blastopore have been prevented from coming together by the material of the injured half; *C*, section of blastula stage of ½ blastomere; *D*, section of ½ embryo like that shown in A. (After Roux.)

half-neural tube; beneath this a round rod (the notochord), and a half archenteron open toward the undeveloped half. At one side of the neural tube and notochord lie the mesoblastic somites of that side. A double sheet of mesoderm extends ventrally over the same side. In all these respects, the embryo is strictly a half-

structure, except that the notochord is a cylindrical rod, i.e., it is round in cross-section.

The accounts of the subsequent development of the half-embryo differ in several important respects. Roux thought that the missing half may often quickly be restored by utilizing the protoplasm and yolk materials of the injured (dead ?) blastomere. He called the restorative process "post-generation." His account of what takes place cannot be accepted as probable in the light of later work. When it is recalled that the changes that are supposed to take place can only be studied by means of sections of embryos that have been preserved, and that no two half-embryos appear to restore the missing part in exactly the same way, it is not surprising with such material that it was difficult to find out what takes place. Roux's account is briefly as follows: The ectoderm of the half-embryo pushes over the surface of the injured half and may ultimately cover it. Where the yolk-cells of the developed half come in contact with the wall of yolk of the other half, a migration of nuclei (and cells?) takes place so that the yolk becomes cellulated. These wandering cells "reorganize" the dead (?) material. Even the mesoderm appears later on the injured half, and it must be supposed that this also comes from the migrating cells, but from which cells is not clear. It is certain, however, that the mesoderm of the developing half could not get across the dorsal midline, since the half-neural tube and notochord would block its passage. Since the notochord is already a cord nothing is needed, unless it be an increase in size, to make it "whole." It is not evident how the missing half of the neural cord is renewed, if in fact it is ever renewed.

There are, in this account, so many statements that do not appear to be in harmony with what is known concerning the early specification of embryonic tissues that the conclusions would have aroused skepticism, even if no further work had been done. Fortunately, the situation has been more carefully examined by later observers (O. Hertwig, Endres and Walter, Laquear, Morgan) whose findings furnish evidence for a very different interpretation of what Roux saw and described as post-generation.

In the first place the work of Hertwig ('93), and later that of Morgan ('02, '04), has shown that the hot needle rarely kills the nucleus of the stuck blastomere, or if the needle is hot enough to do so the other blastomere is also likely to be killed. What

happens in the majority of cases—and these seem to be the ones that produce the half-embryos—is that the protoplasm in the immediate path of the needle is coagulated by the heat. It is this necrotic material that often interferes with the formation of a normal mitotic figure, or else with cellulation of this half. The chromatin is often very imperfectly separated, and irregular clumps of chromatin are found in the protoplasm, as described first by Roux. The irregularities thus begun continue for a time, and normal nuclei are not produced in most cases. In other eggs the division of the nucleus may be sufficiently normal for some of the resulting nuclei to become the centers of cell-divisions in the region of the egg in which they come to lie. Cells form around them, which are added to the half-embryo, and some of these cells may take part in the extension of the ectoderm over the yolk and in the cellulation of the yolk. The necrotic protoplasm, produced by the heat, may prevent the remainder of the blastomere from becoming divided into cells. Under these circumstances the half-embryo does not become complete, but it may appear to be more nearly so than it really is, since the ectoderm of the developed half may also push over the exposed yolk concealing the incompleteness of the parts beneath it.

In other operated eggs the course of events may be different. The division of the nucleus of the injured blastomere may be normal, and the distribution of its later divisions may be less interfered with by the necrotic tissue. The cellulation of the injured blastomere may be delayed, and the uninjured half may proceed to gastrulate before the other half is ready. Whether, after this, the belated half could ever complete its development is very doubtful. If it can do so the rapid restoration of the missing half, that Roux records as taking place, could be explained, but it does not seem probable that the completion ever takes place in this way. On the other hand, if the injured half should become cellulated before the gastrula lip appears, it is possible, in the light of more recent work by Spemann and his collaborators, that this material might be, to some extent, utilized in the somewhat belated development of the injured half of the embryo.

There is indirect evidence, even in the earlier work, that the head of the "half-embryo" is more nearly a whole embryo than a half-structure, and this too is consistent with the more recent

work indicating that, about the invagination to form the archenteron, the overlying layers organize themselves into a whole structure.

The more general results of Roux's experiments may be stated as follows: The injured half is not killed by the hot needle in the majority of cases, but its development is interfered with to various degrees. Its presence, in contact with the developing half, leaves the interpretation of the results uncertain, because the contact of two hemispheres may in itself interfere with the later development of the uninjured blastomere. The results will, therefore, have to be interpreted in the light of other experiments with the frog's egg as well as with those on other eggs. Without further information the results of the sticking experiment prove little more than that the right and left halves of the normal embryo come in many cases from the right and left blastomeres—a result that had already been demonstrated by Newport ('51) and by Roux ('85).

It has been pointed out that while the first cleavage usually passes through the middle line of the crescent, this is not always the case. Sometimes it passes at right angles to the crescent, sometimes through intermediate planes, but the dorsal lip appears in the middle of the crescent, regardless of the position of the first plane of cleavage. As described in an earlier chapter, Brachet has taken advantage of this relation and has examined the effects of injuring the blastomeres in eggs whose first plane of cleavage has one or another position with respect to the gray crescent. Eggs in which the first cleavage had passed through the middle of the gray crescent were injured in one blastomere by a hot needle. Half-embryos, like those described by Roux, developed (Fig. 154*a*). Other eggs, in which the first cleavage was parallel to the gray crescent, were injured by the hot needle in one or the other blastomere. If the blastomere that does not contain the gray crescent is injured, the dorsal lip appears on the uninjured blastomere in the normal position and extends around the yolk as far as the dividing line between the halves. Its closure may be interfered with by the undeveloped half, but at least the anterior end of the embryo develops (Fig. 154*d*). If the blastomere that does contain the crescent is stuck, the opposite blastomere does not produce any recognizable part of an embryo, although it has been doubtfully

stated by Roux that the ventral lip of the blastopore appears under similar conditions. In other cases, the first plane of cleavage may pass in neither one of the two preceding planes, but somewhere between the two, producing one cell that contains most of the gray crescent (including its central part), and another

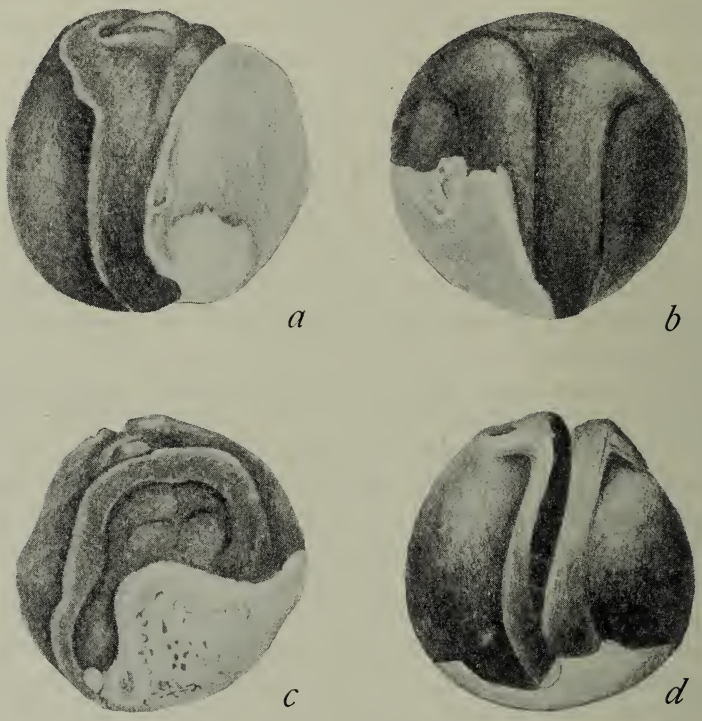


FIG. 154.—*a*, ½ embryo of frog after injuring one blastomere of an egg in which the first cleavage plane was through the middle of the crescent; *b*, a ¾ embryo after injuring the ¼ left posterior blastomere of an egg in which the first cleavage plane was oblique (45°) to the left of the prospective median plane; *c*, ¾ embryo in which the ¼ right posterior blastomere of an egg was injured in which the first cleavage plane was oblique (45°) to the right of the prospective median plane; *d*, the posterior ½ blastomere was injured of an egg in which the first cleavage was frontal. (After Brachet.)

that contains only one side of the crescent. If the half without the crescent is injured an almost complete anterior end develops from the uninjured half.

There is evidence from two other sources that has an important bearing on the interpretation of the half-embryo of the frog. The most direct is that obtained by McClendon ('10). He found

that the contents of one of the blastomeres of the egg of the tree-frog, Chorophilus, could be sucked out by special manipulation with a small pipette, without destroying the remaining one. In most species of frogs this operation does not succeed, because the uninjured blastomere collapses, but McClendon found that the egg of the tree-frog withstands the removal of the contents of one blastomere if the removal is done by degrees. Most of the contents of the stuck blastomere slowly oozes out. What remains may be later withdrawn by a small pipette. The remaining blastomere segments, but the details of the cleavage are not given. A symmetrical gastrula-lip develops that closes as a ring. A bilateral neural plate appears, and a normal whole embryo of half-size results. The experiment shows that the single blastomere of the frog's egg has the capacity to produce a whole embryo as has the isolated blastomere of the sea-urchin, or of Amphioxus. It seems to follow that, in Roux's experiment, half-development is due, in some way, to the presence of the other living blastomere with which the developing half is in contact. The probable interpretation as to how the presence of the injured half affects the results is shown by experiments of another kind. Schultze ('94) found that if the frog's egg is inverted after the two-cell stage, and held in this position, the contents of the two blastomeres becomes rearranged in response to the action of gravity on the egg. The heavier yolk sinks down towards the bottom of the egg (now the black hemisphere, Fig. 155*a*) while the more protoplasmic material rises toward the top of each blastomere.[1]

When the contents of the inverted egg in the two-cell stage rotates, the outcome will depend on the direction taken by the heavier and lighter substances in reaching an equilibrium. If the egg is exactly upside down, with the cleavage plane vertical, the yolk may be expected to sink down along the wall of separation and the lighter parts to pass outwards. This kind of rearrange-

[1] Pflüger ('83) had shown several years before that the contents of the fertilized egg of the frog rotates slowly in response to gravity. Under normal circumstances a fertilized egg, or a segmented egg rotates as a whole within its membranes when disturbed so that its black hemisphere comes to the top, thus showing that the materials of the egg have a sufficient difference in weight to respond to gravity. Special methods had to be devised both by Pflüger and by Schultze to keep the egg from rotating within the membrane when the black hemisphere is turned down.

ment is indicated in Fig. 155*b*. If, however, the egg is tilted somewhat from the vertical the contents may take a different path in each blastomere. The details of the cleavage in individual eggs have not been followed, but the relative size of the cells at the end of the cleavage (Fig. 156*a*) indicates the regional distribution of the protoplasmic and yolk portions of the blastomeres.

Two complete, or nearly complete, neural plates appear in each half (Fig. 156*b-f*). The dorsal surface of one or of both may remain open, forming in each half a partially closed ring (spina bifida) (Fig. 156*c, d, e*), or a complete neural tube may

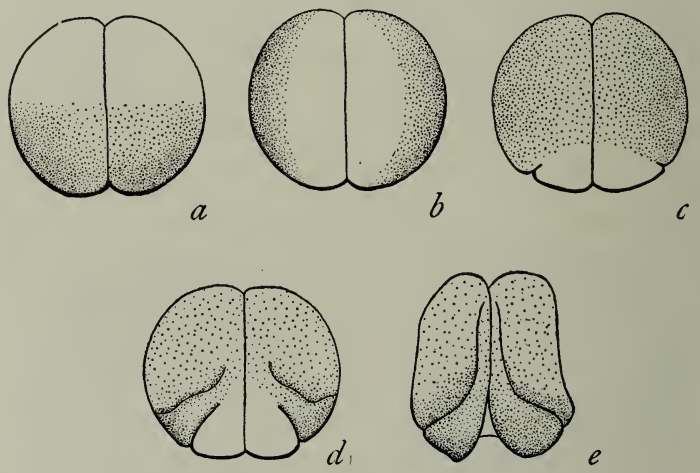


FIG. 155.—Diagrams of inverted two-cell stage of frog's egg. *a*, egg after inversion; *b*, sinking down of yolk along the dividing cell wall; *c*, dorsal lip appearing in each half; *d* and *e*, formation of two embryos, one on each half. This method of gastrulation is not in accord with the process as described by Schleip. (After Durken.)

be formed in each half (Fig. 156*f, g*). In either case there is evidence that a whole embryo tends to be formed from each half. The result establishes the fact that each blastomere may form a nearly complete embryo even while flattened against the other half. The hemispherical shape of the developing half does not appear, therefore, to be an essential factor in determining whether a half of a whole, or a whole of half-size is to develop.

A similar result was obtained by Morgan ('95) in a combination experiment. One blastomere was stuck with a hot needle, as in Roux's experiment. The egg was then inverted (the protrusion of material at the point of injury made permanent inver-

sion possible without resorting to other means); the uninjured part segmented, gastrulated and formed a whole embryo (more

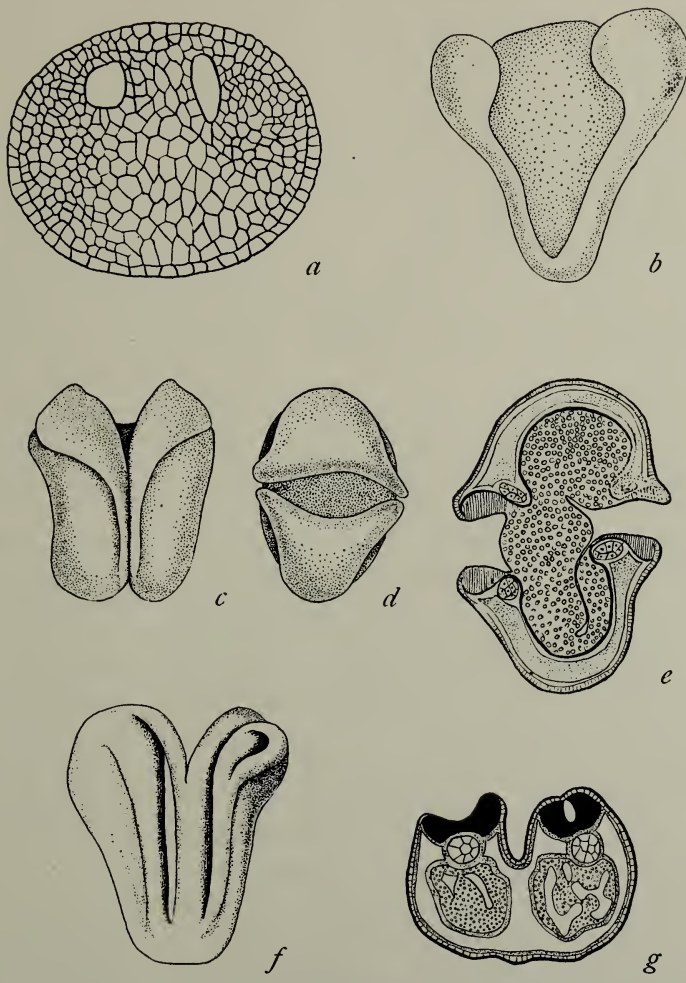


FIG. 156.—Embryos from inverted two-cell stage of frog's egg. *a*, section of cleavage stage; *b*, two whole embryos on opposite sides of egg; *c*, two embryos back to back; *d*, same from anterior end; *e*, cross-section of last. In these embryos the half-neural plates at the sides of the two embryos have not united into single neural tubes as in the typical duplicitas cruciata, and the embryos are interpreted as two whole embryos with spina bifida. *f*, two embryos side by side; *g*, cross-section of such an embryo (After Wetzel.)

or less complete) while in contact with the other half from which it was sometimes constricted. Here again we must suppose that

the contents of the uninjured blastomere rotated away from the normal position. A normal, whole embryo of half-size resulted. As stated above, results of this kind, taken in connection with McClendon's experiment, show that the behavior of the first two blastomeres (whether into a half or into a whole structure) is determined by the distribution of the materials within each blastomere, and not, in a physical sense, by the form of the resulting mass of cells derived from each.

In the normal egg like regions are present on each side of the prospective middle plane. All of the material of both sides, derived from the gray crescent turns in during gastrulation to produce a single structure (the archenteron); but after inversion the crescent region in each blastomere, from which the dorsal lip is to develop, may become separated. In consequence two dorsal lips might be expected to develop, each independent of the other and two independent archentera result. Each then becomes the center of a whole embryo of half-size. This is little more than a restatement of the facts, and cannot pretend to be an explanation of the mechanics of the "organizing" agents.

Some of the embryos described by O. Schultze ('94), appear to have two entire heads with one body (duplicitas anterior); others are complete, but united by the dorsal surfaces; others lie side by side; while still others, that have been later recognized as Janus embryos (duplicitas cruciata), are united back to back in the head region. The last type if split open along the mid-dorsal line resembles spina bifida embryos, but should the half-neural folds on each side unite to form a single tube a true Janus formation would result. It seems probable that some of the embryos found by Schultze were of this kind, although he did not specifically recognize them as such.

Schultze's experiment has been repeated by Wetzel ('95, '96) on the frog (Rana fusca); Chiarugi ('98) on Salamandra perspicilla; Tonkoff ('00, '04) on Triton cristatus; and by Schleip and Penners ('25) on Rana fusca.

The experiment when repeated by Wetzel added further information in regard to the internal conditions of some of the inverted eggs during the early stages. He found, for instance, in some of the later segmentation stages that the light-colored cells at the top of the egg were smaller than those below, and that two separate blastocoels are present (Fig. 156*a*). He

showed, as Schultze had already done, that the results were due to the rearrangement of the cytoplasm in each of the two cells, the lighter material flowing in each toward the top, the heavier sinking to the bottom of the blastomere. The different kinds of embryos (Fig. 156) were due, he thought, to the different ways in which the redistribution of the materials of the egg had taken place.

More recently Schleip and Penners ('25) have made a more detailed analysis of embryos obtained by inverting the egg, and have shown that many of the embryos that Schultze did not recognize as such may be classified as Janus-like forms. Moreover, by a more careful scrutiny of the changes that take place in eggs, in which the position of the gray crescent is known, some further information has been obtained relating to the internal changes that occur after inversion. These results will be described in the next section.

The Development of Janus Embryos (Duplicitas cruciata) of the Frog

The occurrence of "double monsters" united with their "faces" turned in opposite directions has been known for a long time. The earlier and more familiar cases of Janus embryos are human or mammalian, whose mode of origin is uncertain. From the method of development of the mammalian embryo it is improbable that they originate in the same way as those here described under the same name. In these cases the special point of interest is not so much that the two heads face in opposite ways, but the fact that the two bodies are each a composite of the right side of one embryo and the left side of the other. The origin of the older cases was often discussed, but there was little basis for discussion until the development of whole embryos from isolated blastomeres became known, and the development of two embryos after incomplete separation of blastomeres had been worked out. More definite information came, however, with Schultze's ('94) discovery that by inverting the egg of the frog in the two-cell stage double embryos could be artificially induced in relatively large numbers, although he did not interpret any of them as Janus embryos. The more recent work of Spemann ('18), and that of Hey ('11) on Triton, and Schleip and Penners ('25, '26) on the frog's egg, have given results that go far toward explaining the origin of certain kinds of these duplicate embryos.

A typical Janus embryo, Duplicitas cruciata typica, of the frog is drawn in Fig. 157*a*. It has two anterior ends (head-folds) pointing in opposite directions. To the right and left of each head, a half-neural fold extends around the sides; the right half of one embryo and the left half of the other running parallel to each other and uniting to form a whole neural plate on each side.

A modified type of Janus embryo is represented in Fig. 157*b*. The two anterior ends are not exactly opposite, but extend forward in the same general direction. Correspondingly the two neural folds on one side are less developed than those on the other. If such an embryo should develop further it would appear superficially to be a double-headed embryo (duplicitas anterior), while in reality it is a Janus type with one-half of the body more or less suppressed.

A third type is shown in Fig. 157*c*. This embryo can perhaps also be referred to the Janus type, as Schleip and Penners point out; the lateral materials that have come together on the less developed side having turned towards the more developed side, inserting themselves, peninsula-like as it were, between the two widely separated halves of the other side. Consequently the left half-neural plate becomes the partner of its own right half-plate rather than the partner of the right half-plate of the other side. Similarly for the right half-plate. It follows that the two inner half-folds of the "peninsula" that have been inserted between the two outer half-folds are opposed to each other in reverse orientation, and under these circumstances would not be expected to combine into a single neural tube as in Janus-formation, but become rather the complements of their "own" folds. It is questionable, therefore, whether there is any advantage to be gained by comparing such an embryo with the Janus type, unless it can be shown that in such a case as this there are not two reversed half-neural plates, but a single plate.

If such a condition as that shown in the last figure is carried one step further, an embryo, like that of Fig. 157*d* would be produced. Two nearly whole embryos appear lying side by side. The peninsula-like tongue of material lying between them is interpreted by Schleip and Penners as the half-right, half-left extension of the two heads between the two half-neural plates of the opposite side. The half-neural folds belonging to each head

are supposed no longer to be complements of the other halves belonging to the other head, but here back to back run parallel to unite with their own halves. (Compare with Fig. 156*f*, *g*.)

In the last two cases it may seem doubtful whether there

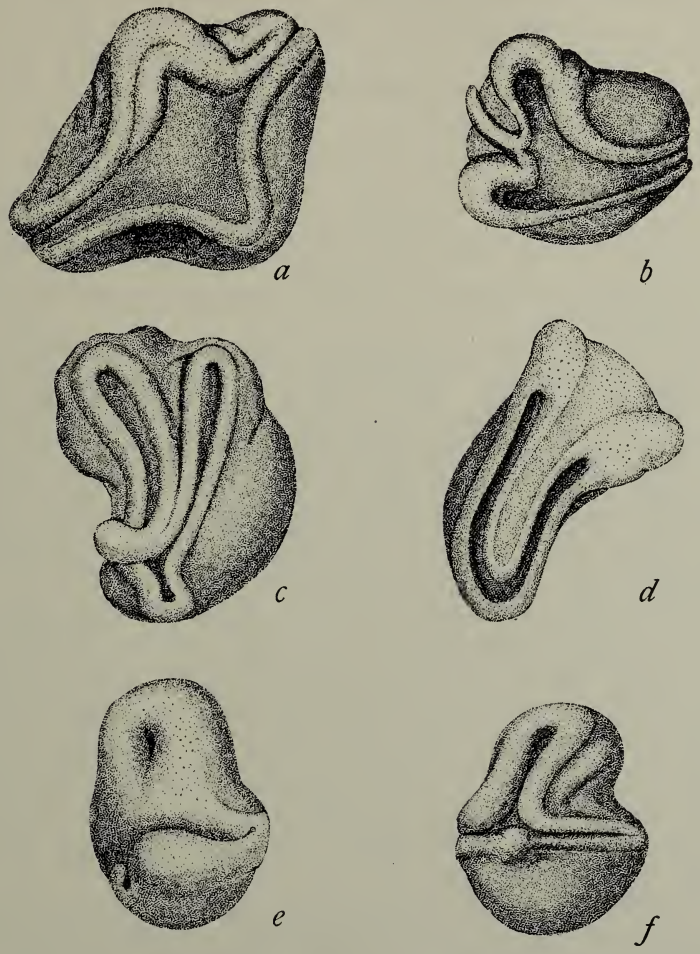


FIG. 157.—Development of two embryos on inverted frog's egg in two-cell stage, interpreted as duplicitas cruciata typica. (After Schleip and Penners.)

is any advantage in trying to interpret such embryos in terms of the typical Janus type. It probably would be more profitable to follow in each case the course taken by the advancing lips of the double dorsal lip (or two dorsal lips) of the blastopore,

and the method by which the materials in their vicinity become involved to produce, as far as the conditions allow, two whole embryos. To some extent this has been done in a later study by Schleip and Penners ('26), and will be considered below.

Other more extreme types are also referred, by Schleip and Penners, to modifications of the Janus-form. Such a type, for example, as that shown in Fig. 157*e*, is interpreted as having one well-developed anterior end, whose neural plate divides further back into right and left halves. Opposite this head there is an imperfect anterior end of the twin, whose half-neural plates likewise extend right and left to run parallel to those of the other more complete embryo. A more developed embryo of the same sort is shown in Fig. 157*f*. In this the minor head is little more than a knob, but its lateral extensions seem to be equal to those of its co-twin.

A number of other twin monsters are described and analyzed by Schleip and Penners. Their description may help to interpret many of the curious monsters that owe their origin to the development of twin embryos from united blastomeres. It should be added that, while Schleip and Penners have been able to refer a large percentage of the twin embryos arising from inverted eggs to the Janus type, they recognize also other monstrous twin-forms referable to other types of development.

An important contribution to the subject has been made by their more exact observations of the early stages of the inverted eggs. Eggs, reversed 10 minutes, 30 minutes and then every half hour for three to three and a half hours after fertilization (when the first cleavage had not begun), gave normal embryos even although the yolk had been observed to shift.[2] On the other hand, eggs that were compressed and turned upside down after the two-cell stages frequently gave twins. The following table (Table XXIII) gives the results.

It is obvious that even after the "yolk" has reversed (as determined by observation), a whole embryo often develops, as in Pflügers and Born's experiments. This is also true after the 4-cell stage. The table also shows that after the 4-cell stage and even the 8-cell stage is reached twin embryos may appear. It may be recalled that even after the 8-cell stage the gray

[2] One pair of twins united ventrally, was obtained in the set that had been inverted 10 minutes after fertilization.

TABLE XXIII

Cleavage Stages of the Inverted Egg	Number of Normal Single Embryos	Number of Double Embryos
Second cleavage beginning.....	34	13
Second cleavage half around egg....	40	4
Second cleavage nearly or quite finished	76	5
Third cleavage beginning.....	8	4
Third cleavage just finished.....	6	1
Sixteen-cell stage.....	35	0

crescent is still contained in two blastomeres as it is in the 2-cell stage.

Eggs in which the gray crescent was clearly visible were more closely followed in some of the inversion experiments. The position of the first cleavage was marked and also the plane of symmetry of the gray crescent. In one experiment in which the first cleavage was in the median plane there were 8 whole embryos and 28 twins. In other eggs in which the first cleavage was at right angles to the crescent (frontal) there were 6 whole and 4 twins. In another experiment the former gave 66 whole and 43 twins, the latter type 28 whole and 31 twins. The results seem to mean, if I interpret them correctly, that, whether the first cleavage divides the gray crescent symmetrically or not, twin embryos may arise. But even if the first cleavage does not divide the crescent, the second cleavage may do so, and if the rotation of the interior should continue and if the results are due to this, twins might still be expected. There is, however, still another question involved here. If the first cleavage is at right angles to the plane of symmetry of the crescent, i.e., if all the crescent material is contained in one blastomere, an embryo (or twin ½ embryo) might be expected to come from the material of that blastomere only, i.e., from that side of the egg containing the crescent, and no embryo from the opposite blastomere lacking the material of the crescent. When the egg of Triton is constricted by a thread only one embryo arises when the first cleavage is at right angles to the median plane, and in all probability this would be expected to hold for the frog also. I do not understand that these experiments of Schleip and Penners have shown that under such conditions each blastomere develops an embryo, for, as mentioned

above, twins might possibly still be expected with the second cleavage plane as the dividing plane.

Schleip and Penners state emphatically that the dorsal lip in the inverted eggs that will give rise to twins, probably always appears in the region of the gray crescent of the egg. They interpret this to mean that the material of the crescent takes no part in the movements following inversion of the egg. They also state that when the dorsal lip appears it is a continuous invagination. They were not able to explain the connection between the inversion of the egg and the development of two embryos and are skeptical concerning Wetzel's evidence that two dorsal lips appear in consequence of the redistribution of the contents of the blastomeres. Nevertheless while it may be true that the material of the crescent is less dislocated than other parts of the egg, it must appear probable, even from the evidence of Schleip and Penners, that some disturbances or dislocations of this material occur with sufficient frequency to produce two organization centers, each giving rise to an independent dorsal lip.

The bearing of these experiments on the interpretation of Roux's half-embryos is significant. In his half-embryos, half of the material of the gray crescent is thrown out of action, the other half becomes organized into a dorsal lip. It is customary to regard this as half a dorsal lip, but there is no evidence that such is the case. In fact, the presence of an entire head in the half-embryo indicates that in front of the dorsal lip a whole structure is in process of developing, but on one side the extension of the lateral lip is prevented, and this half of the embryo fails to be produced.

A more detailed attempt to explain the origin of Schultze's double embryos has recently been made by Schleip and Penners ('26). They point out that gastrulation of these embryos shows a wide departure from the normal process. Their explanation rests on the assumption that the inturning of the yolk mass is the essential phenomenon of gastrulation, and that the rest follows as a consequence. In the inverted egg the yolk has sunken down along the first cleavage plane, and has, thereby, become somewhat separated into two independent masses. Each mass sinks deeper into the interior at the time when the blastopore rim appears on the surface as a single line between the two yolk masses, and on the side of the original gray crescent. The sur-

face-cells on each side of this rim now turn under to the right and left, following the yolk movements. Two invaginations of endo-mesoderm result in opposite directions. Each acts as an organizator, and calls forth the anterior end of an embryo, the two standing opposed to each other. At their posterior limits each divides right and left in those cases where a typical Janus embryo will result, and if, then, the halves unite to form a common trunk, on each side, the typical cruciata type develops. It seems rather questionable to lay so much emphasis on the yolk-cells rather than on the cells derived from the crescent. It appears more probable that the crescent cells may be the initiating influence in the gastrulation of the inverted embryos, as they are in the normal development. If, owing to the inversion at the 2-cell stage, the crescent material is also drawn out into a line, more or less at right angles to its earlier position, i.e., along the first plane of division, such a redistribution fits in very well with the subsequent appearance of a "vertical" rim representing the beginning of inturning.

The Development of Partially Separated Blastomeres of the Eggs of Salamanders

Many observations and experiments on the eggs of several long-tailed amphibians, especially on the eggs of Triton, have shown that, *in most cases*, the second and not the first plane of cleavage corresponds to the median plane of the body.

Hertwig in 1893 tied a loop of silk thread around the egg of Triton in the 2-cell stage, attempting in this way to separate the two blastomeres, and although he did not succeed in doing so, he found out that the median plane of the embryo stood, in the eggs examined, at right angles to the constricting thread, and hence at right angles to the first plane of cleavage. In the same year von Ebner reported that, as a rule, the first plane of cleavage of the egg of Triton separates the anterior and posterior parts of the embryo, but that exceptionally the first cleavage separates the right and left halves of the embryo. Endres ('95) on the other hand reached the conclusion that normally the first cleavage plane is the median plane, although exceptionally (under the influence of experiment) the second or third cleavage planes may take the place of the first cleavage. Herlitzka ('95, '97)

appears also to have thought that the first plane corresponds to the median plane as in the frog. The far more extensive and exact experiments of Spemann have shown clearly, however, that in most cases the second plane is the median plane. His conclusions rest on a large number of experiments with the egg of Triton of several species. The eggs were constricted by a hair. He found that in ⅔ to ¾ of the cases the second cleavage of T. taeniatus corresponds to the median plane while in ¼ to ⅓ of the cases the first plane corresponds to the median plane of the embryo.

Endres ('95) after constricting the egg with a thread, injured the connecting piece by sticking with a hot needle. The first two blastomeres were separated in this way. His brief preliminary account does not make clear what followed. Spemann says that the results appear to be similar to his own, in which one of the two blastomeres produced a normal embryo, the other an atypical structure that went no further than the gastrula stage.

In his first paper Herlitzka reported that, in four cases in which the constriction was in the plane of the first cleavage, whole embryos came from the blastomeres. They developed as far as the gastrula or neural fold, or neural tube stages; but as Spemann points out his evidence fails to show that in these four cases both halves produced whole embryos. Herlitzka's figures show that, in all four cases, only one of the two blastomeres really reached the completed medullary plate stage. Later Herlitzka obtained complete embryos from each of the first two blastomeres of the same egg. This is possible, according to Spemann's results, only in cases where the first cleavage happened to correspond to the future median plane of the embryo. Spemann has also obtained in two cases out of three, in which the constriction separated completely the first two blastomeres, two whole normal embryos in each case. In the third case, one blastomere gave a whole embryo, the other an atypical structure. Spemann also obtained a large number of double embryos from eggs when the constricting thread did not cut the embryo completely in two. In those cases in which the blastopore appeared in one only of the two halves that half produced an embryo, while the other half made only a gastrula-like structure. It is evident in this case that the first plane of cleavage lay at right angles (or thereabouts) to the median plane. It does not follow, however, that the "anterior

blastomere" produces exactly only the anterior end of whole size, nor does it follow that it forms exactly a whole embryo of half size. In normal whole development some of the materials of the anterior blastomere may be transferred across the imaginary line of the first cleavage to contribute to the material in the other blastomere and produce a part of the posterior end of the embryo. The failure of the posterior blastomere when isolated to produce an embryo may be due to lack of materials, but possibly also to the absence of the dorsal lip that initiates the gastrulation process.

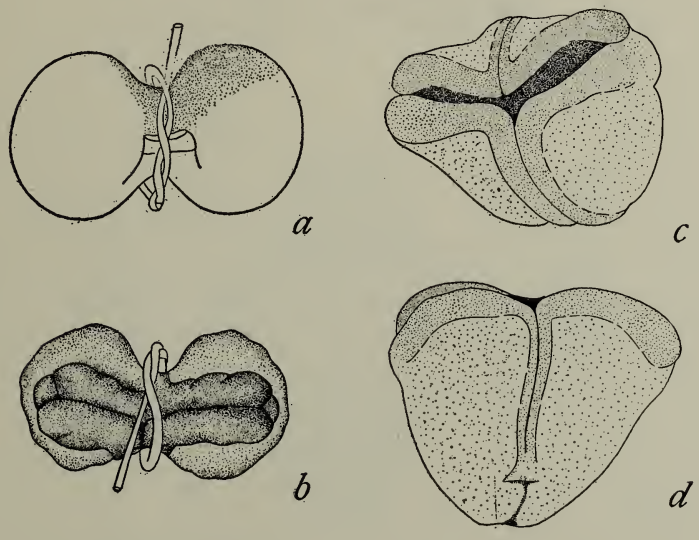


FIG. 158.—*a*, constricted egg of Triton in gastrula stage; *b*, an anterior end has developed on each half; *c*, embryos of same in which two whole anterior ends have developed on each half and the lateral extensions of each to the right and left have united to form two complete neural plates, one on the right, the other on the left side; *d*, side view of last. (After Hey.)

This seems probable from the experiments in grafting that Spemann has made showing that almost any region of the egg may make an "embryo" (medullary plates, etc.) if a dorsal lip be grafted into it.

In other cases in which the blastopore is later found to be bisected by the constricting thread, i.e., when it lies in the connecting piece, double embryos were obtained; often a double-headed form with a single trunk. Here each half tends to form a whole structure at the anterior end. If the constricting hair

had completely separated the blastomeres each would, no doubt, have formed a whole embryo. Some of the peculiarities of the double-headed embryo will be described in another connection.

As stated above, when the first cleavage of the egg of Triton corresponds to the future median plane of the embryo, it has been shown by Spemann ('03) that a constricting thread around

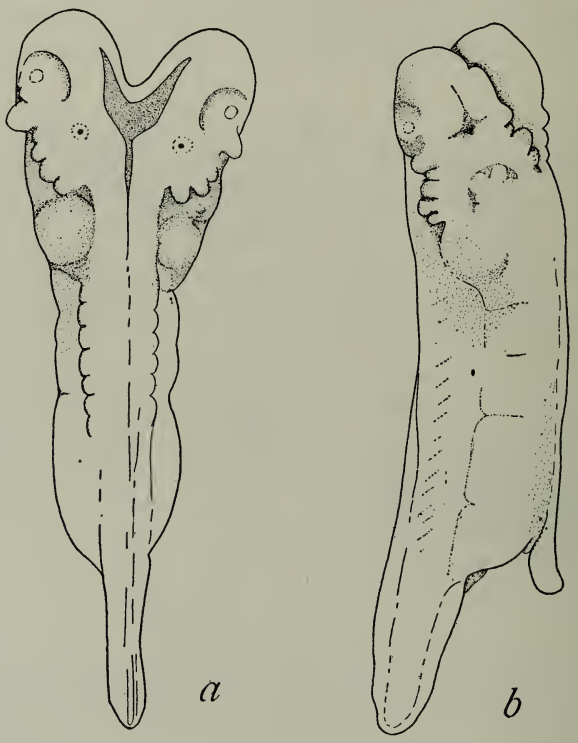


Fig. 159.—Duplicitas cruciata embryo of Triton that has developed from an egg constricted in the median plane as in Fig. 158. *a*, from the lateral side; *b*, from the ventral side of one embryo. (After Hey.)

the egg in this plane often leads to double-headed "monsters." Under these circumstances the material of the neural plate develops into two whole heads which lie side by side to the right and left of the middle plane of the body (Fig. 73). Under similar conditions there is also occasionally formed a Janus-type. Several of the Janus forms have been described in detail by Hey ('11) whose work was done under Spemann's direction. If, as shown

in Fig. 158*a*, *b*, the constricting threads are tightened during the gastrula stage, so that the constricted egg has somewhat a sand-

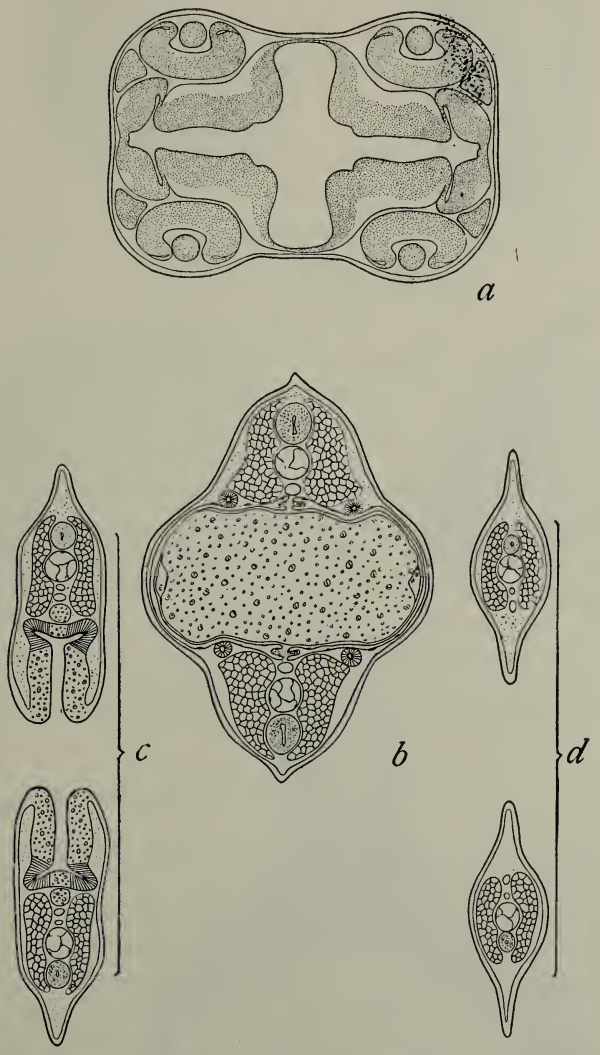


Fig. 160.—Diagrammatic cross-sections of the embryo shown in Fig. 159. The median plane of the two heads in *a* is at right angles to the two median planes of the trunk regions, as shown in *b*, *c*, *d*. (After Hey.)

glass form, two anterior ends often develop at right angles to the thread (Figs. 158*b*). The two brains (that later develop

from these plates), and the two heads of the monster, are, so to speak, back-to-back (Fig. 158*c*, *d*), but when the halves of the plates reach the constricted region, the left half (neural fold) of one head and the right half of the other unite to form a single neural tube. Similarly, on the other side of the right half (neural fold) of one head and the left half of the other unite to form another neural tube.

A diagram representing cross-sections (Fig. 160) through an older Janus embryo (Fig. 159) will help to make these relations clearer. The first cross-section (Fig. 160*a*) is taken through the brain of the two heads. The brain appears in the middle of the section. The four eyes appear at the corners of the section. The two lobes of the brain that lie above and below in the middle line represent two sides of the neural tube that are continuous at lower levels with the dorsal tubes of the embryo. The second section, *b*, is taken through the middle of the embryo. Two neural tubes are present, two notochords and a double set of corresponding mesoblastic somites. There is a common central yolk-mass. The two sections, *c*, through the anal region, are placed in a position corresponding with *b*. In the more posterior regions (sections *d*) the tails are free. In other cases one embryo may be more complete than the other due to the constricting thread not being exactly in the middle line. The dorsal cord may be imperfectly developed on one side or even absent, and the notochord and somites may be similarly deficient.

The Formation of Janus Triton Embryos by Grafting

It had been shown by Spemann ('16, '18) that it is possible to cut in two vertically the gastrula-stage, and then to reunite halves containing half-dorsal lips of the blastopore in different relations to each other. If the two half-gastrula lips are brought side by side a single embryo may result. If, on the other hand, each half contains the whole dorsal lip (the top-half of each gastrula having been cut off as shown in Fig. 161*a*) and these two gastrula-halves are united in such a way that the anterior end of each dorsal lip is turned at an angle away from the other dorsal lip (as shown by the arrows), an embryo results with two heads and a single body (Fig. 161*b*, *c*).

If two halves from different gastrulae are united as shown

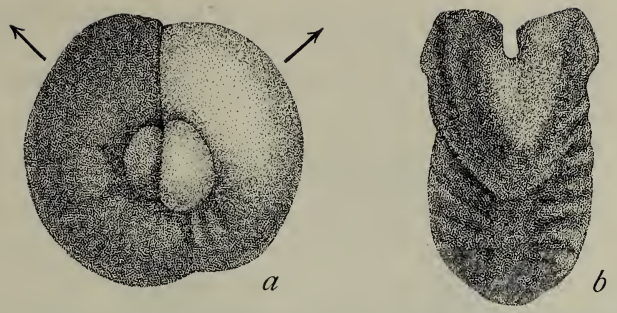


FIG. 161.—*a*, union of two gastrula halves with the dorsal lips turned at an angle away from each other; *b*, an embryo formed from one such union with two heads and a single trunk. (After Spemann.)

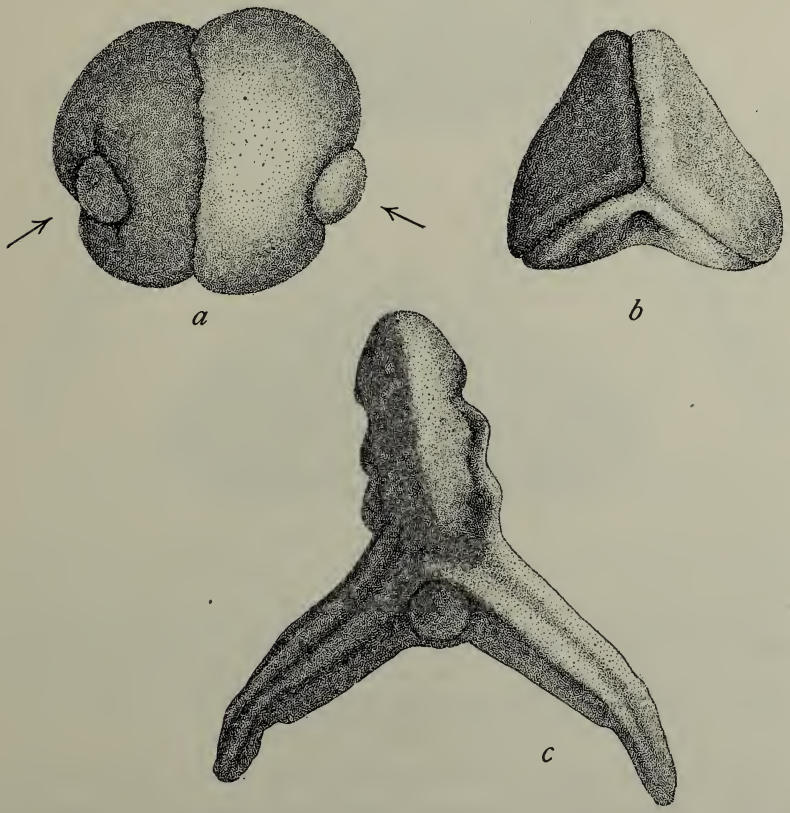
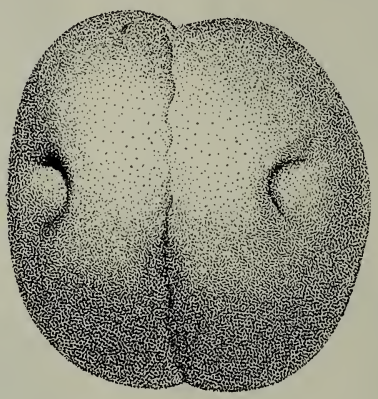
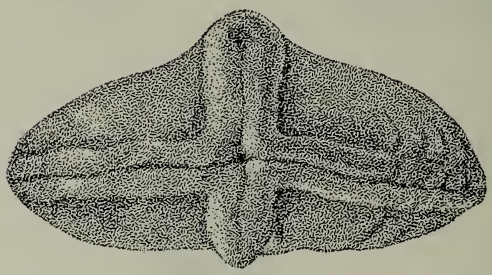


FIG. 162.—*a*, union of two gastrula halves with the dorsal lips turned at an angle towards each other, as indicated by the arrows; *b*, a neurula stage resulting from such an embryo; there is a single head formed by the union of materials from each gastrula as indicated by the color differences of the two united gastrulae; *c*, an older stage of the same embryo with a single, combination-head, and two independent trunk regions, one for each half. (After Spemann.)

in Fig. 162*a*, with the anterior end of each dorsal lip directed obliquely towards the other one (as shown by the arrows) an embryo develops with a single head and two separate bodies (Fig. 162*b*, *c*).



a



b

Fig. 163.—*a*, union of two gastrula halves of Triton with the dorsal lips directed exactly towards each other. *b*, embryo from same; two heads are present, each formed out of materials of both halves and turned in opposite directions, and two posterior single trunk regions, one from each half. The median plane of the heada is at right angles to that of the two trunk regions. The result is a typical cruciats embryo. (After Spemann.)

If two halves from different gastrulae are united as shown in Fig. 163*a*, with the anterior end of each dorsal lip opposite the other one, a Janus embryo may develop with two heads, each composed of parts from each half, and two bodies, each from only one of the gastrulae. The median plane of the heads stands at

right angles to the median plane of the trunks (Figs. 163*b* and 164*a*, *b*).

The method of development of the Janus embryos has been further studied by Miss Else Wessel ('26) in Spemann's laboratory. Two lower halves of gastrulae of Triton cristatus (one of them stained in Nile-blue) were united as described above (Fig. 163), so that the dorsal lips were opposed. In some cases the two dorsal lips were near the plane of union, in which case the anterior materials soon came in contact and the two anterior ends (at right angles to the trunk) are relatively long; in other

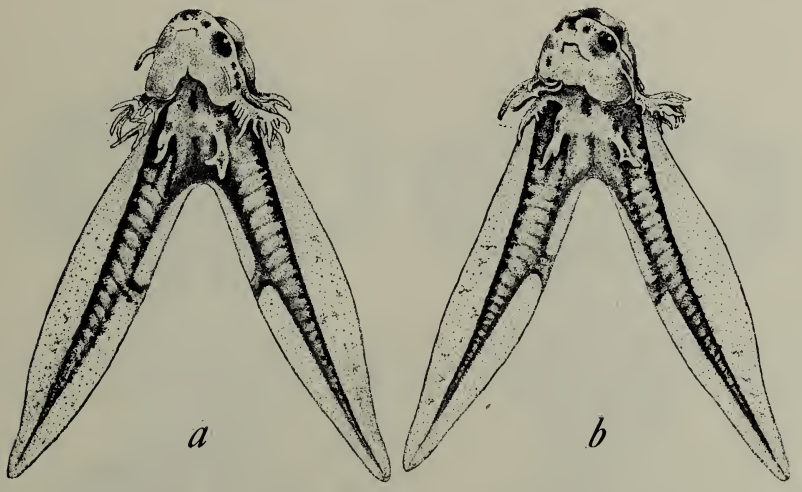


FIG. 164.—Opposite sides of older cruciata embryos of Triton formed by union of two half-gastrulæ, as shown in Fig. 163. (After Spemann.)

cases the two dorsal lips were further from the plane of union and only the anterior ends of the embryos came together, where they might form a slightly Janus-like head or merely come in contact. A few of these embryos will serve to illustrate the kinds of results obtained.

A young stage of a typical Janus embryo is shown in Fig. 165*a*, *b*, *c*. The dorsal aspect of the embryo (*a*) shows the crossing point; one anterior composite end, formed of halves from each embryo, lies above in the figure, the other below. To the right and left the trunk region of each embryo is also seen. Each is formed only out of the material of its half. In *b*, a side view of the composite head of one side is shown with the trunk region

below, and at the edges; the other composite head is shown in *c*, which is less well developed than is the composite head of the other side. A later stage of this embryo is shown in Fig. 166*b*.

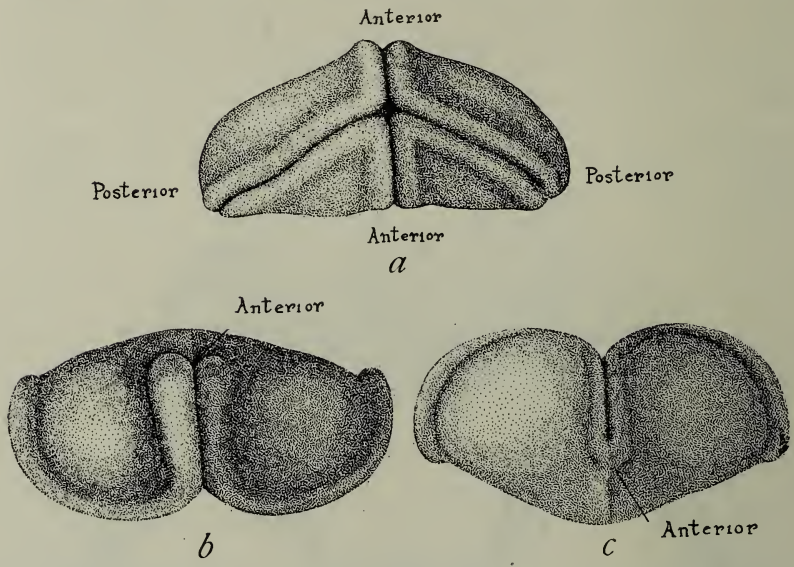


Fig. 165.—Another cruciata embryo formed by the union of two half-gastrulæ united as in Fig. 163. *a*, neurula stage as seen from dorsal aspect; *b* and *c*, side views of same, showing the union of the two half-heads into a single structure. (After Wessel.)

Fig. 166.—Another embryo formed in the same way as the last (Fig. 165). *a*, neurula-stage as seen from the dorsal aspect; *b*, later stage of the same as seen from one side; the union of the two halves to form a single head on this side is shown. (After Wessel.)

For comparison the dorsal view of the preceding stage is shown (to the left). In the older stage, *b*, one of the composite heads is turned towards the observer, while the two single trunks extend right and left.

A later stage of double embryo, formed by the union of two opposed gastrulae, that is not a Janus, is shown in Fig. 167. In this case each gastrula has formed a complete whole embryo, except in so far as they are united ventrally throughout their length, and consequently the hearts lie on the two sides. A cross-section through the anterior end shows that in each two optic vesicles are in process of forming from each brain. A cross-section of a somewhat similar embryo is seen in Fig. 170, taken through the heart region. On each side (right and left of the figure)

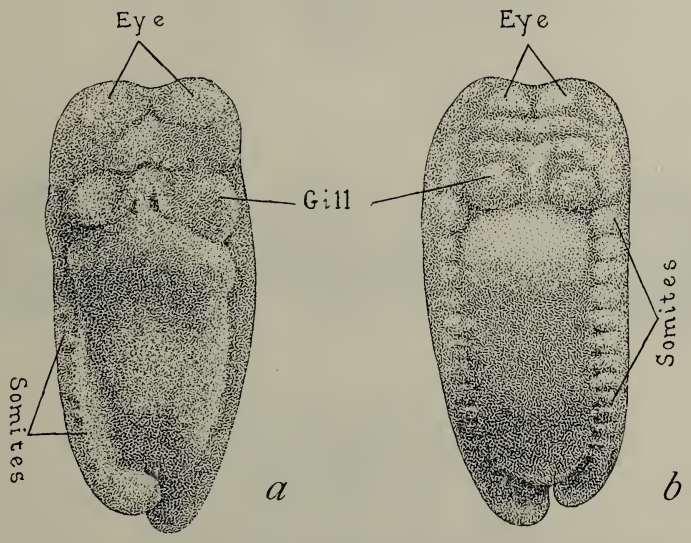


FIG. 167.—Opposite sides of a compound embryo in which only the most anterior parts have united; the remainder of each embryo has come from each half. (After Wessel.)

the nerve-cord, notochord, mesoblastic somites are present. There are two separate digestive tracts. The beginning of two hearts is seen above and below in the figure. Since the embryos are united by the ventral surfaces, where the heart of each belongs, the two hearts come to lie outside the middle line.

A Janus embryo, in which the two anterior heads and trunk regions (which are composites) are long and the single tail ends are relatively short, is shown in Fig. 168. Above, the embryo is seen from one side, showing one anterior composite head and trunk; below, the opposite side is shown, where the other anterior composite head and trunk are present. In the middle of the group

the embryo is seen from the posterior end, where the crossing to form the two single tails is shown. To the right and left are two other figures that show the anal ends of the right and left sides.

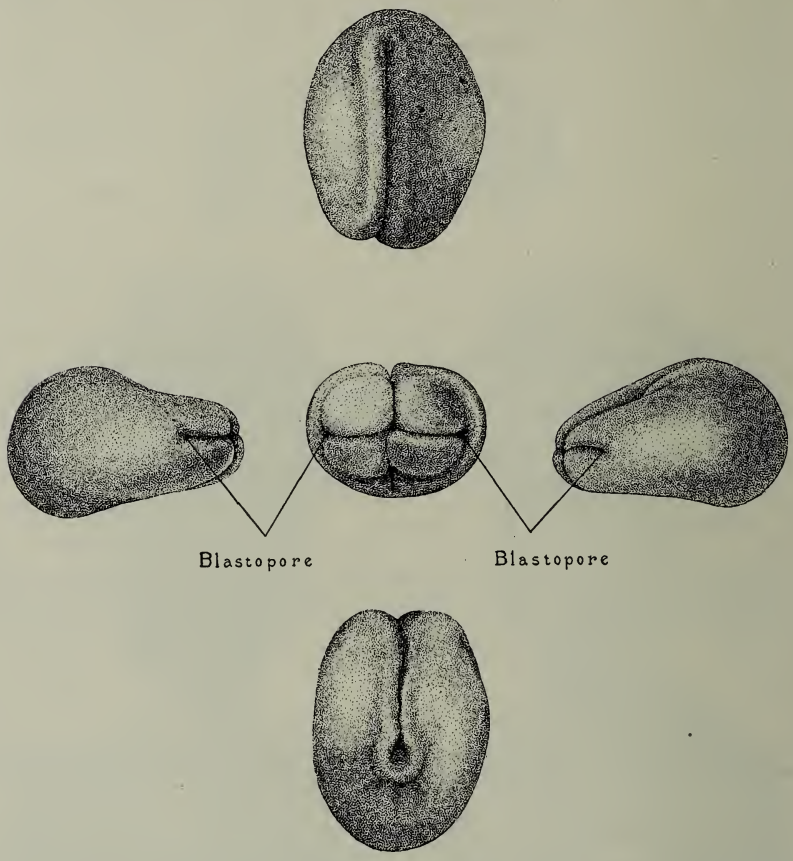


FIG. 168.—A cruciata embryo formed by the union of two gastrulæ (as in Fig. 163) in which the heads and trunk regions of each embryo have been formed by the union of materials from the two halves, while only the two tails have been formed by each half alone; above, head-end of one embryo; below, head-end of other embryo; center, posterior region viewed from the end; left and right, side-views of the tail region formed out of each half alone. This embryo is a cruciata embryo, and only a small egion of the tail is single, the two trunks and heads are combinations. (After Wessel.)

An older stage of this embryo is shown in Fig. 169*a*, *b*. One side, *a*, is better developed at the anterior end than is the other side, in which the brain is imperfect. The trunk region is better developed on the less perfect side than is the head region.

These Janus embryos can be explained as the results of an apposition of the materials from the two dorsal lips at the plane of juncture. The simplest interpretation is that, as the chorda-

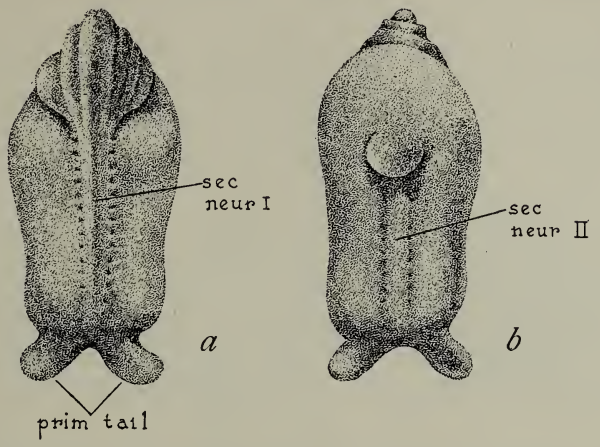


Fig. 169.—A later stage of the Janus embryo drawn in Fig. 168, as seen from the two sides. The embryo on one side, *b*, is imperfectly developed at the anterior end. The median plane of the trunks is at right angles to that of the two tail-ends. (After Wessel.)

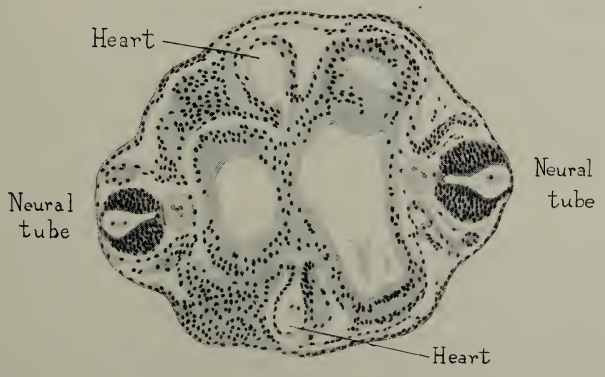


Fig. 170.—Section through a Janus embryo drawn in Fig. 169. The two neural tubes and notochords are on opposite sides, the two digestive tracts are fused in the middle line so that the two hearts are crowded out to the two sides. (After Wessel.)

mesoderm is inturned and extends forward from each dorsal lip, the two forward-growing tongues of material meet at the juncture, and then divide right and left. The result would lead to the material of one tongue on the right side uniting with that of

the left side of the other tongue to form a composite anterior end, and similarly on the other side. The neural plate develops in conformity with the presence and location of the chorda-mesodermal materials. The most surprising result is the coördination shown by the materials from the two embryos where they come together at the junction and divide, passing to the right and left to unite with similarly divided materials of the other half—the two halves now forming a head on each side that is composite in origin. Should the materials not meet and divide symmetrically, one side might be defective, but the other side, that would then presumably sometimes have an excess of materials, develops nevertheless as a single structure. These results are in accord with many other experiments on Triton that show a wonderful plasticity of the organizing materials in certain well-defined directions. No doubt the processes involved are only an extension of the same phenomena taking place in the normal embryo.

Injury to One Blastomere of the Ascaris Egg

Boveri had found by exposing one of the blastomeres at the 2-cell stage of Ascaris to ultra-violet rays that it was possible to injure that blastomere without affecting the other. At his suggestion Miss Stevens ('09) carried out a more extensive study of the effects produced, and Boveri ('10) has also briefly commented on the results. Schleip ('23) has later studied the effects of much more intensive rays on the egg of Ascaris, using the method of Tschachotin ('12, '21).

Boveri, according to Stevens, had found that when the AB blastomere is injured the P_1 blastomere follows the type of cleavage that it would have undergone under normal conditions (see Fig. 14). Similarly when the P_1 is injured, the AB blastomere goes through its characteristic divisions. Stevens repeated and confirmed these observations. The AB cell (after injuring the P_1 cell) divides regularly into two, four, and eight cells, etc. Later the ectoderm-like cells arrange themselves into a hollow blastula (Fig. 171*a-c*). They never produce the characteristic organs of an embryo, unless the regular epithelial-like arrangement of the ectoderm cells into a hollow sphere may be identified as the skin of an embryo. The injured blastomere remains alive, and in the cases chosen for study did not divide, although in other

cases it may divide much later, and then irregularly. The dividing cells of the developing half appear to lose their contact relations with the injured cell, reacting only to contact with their normal fellows.

The cleavage of the P_1 blastomere is also closely similar to its cleavage in the normal egg. It first divides into P_2 and EMST cells, and then, two cells are said by Stevens to change their shape and position, as they do in the normal embryo, to form

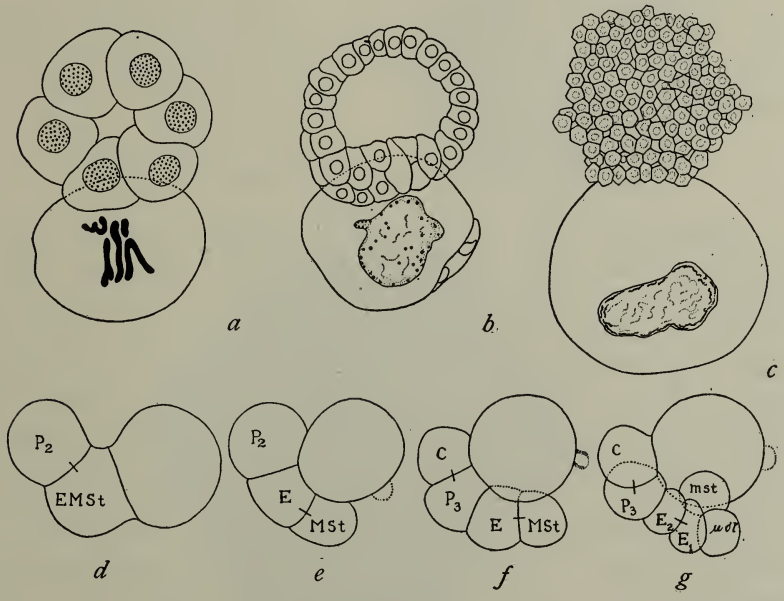


FIG. 171.—Eggs of Ascaris megalocephala in which one blastomere had been killed by ultra-violet light. *a–c*, later development of AB blastomere; *d–g*, later development of P_1 blastomere. (After Stevens.)

the trapezoid stage (Fig. 171*d-g*), but whether their contacts with the injured cells play any rôle in the change, or merely serve as orienting points is not clear from the description. Each of these cells next divides in such a plane as to give a row of four cells, C, P_3, E, MSt (Fig. 171*f*), as in the corresponding normal cleavage. The following divisions (Fig. 171*g*) also resemble those of the normal embryo. In the solid mass of cells that results from the division of this blastomere, the cells characteristic of endoderm, mesoderm, germ-cells, stomodaeum and tail-cells can

be recognized, but in the absence of the covering ectoderm the mass remains incomplete and does not form an embryo.

When three blastomeres of the 4-cell stage are injured the remaining one, whichever it may happen to be, forms the same group of cells as it does in the normal embryo. When only one blastomere of the 4-cell stage is injured, the other three may form approximately a ¾ embryo which is defective only for the group of cells that would have come from the undeveloped blastomere.

These experiments show that there is little self-regulation in the Ascaris cleavage. Each blastomere contains within itself those factors that determine its fate, although there is evidence that the contact of the cells determines to some extent the shape assumed by the whole mass.

Janus Embryos of Tubifex

Amongst the abnormal embryos of Tubifex rivulorum, that are often found in the capsules laid by this worm, there occur, extremely rarely, double embryos that belong to the Janus type of embryo showing Duplicitas cruciata. Penners ('22, '24) has made a careful study of the development of these interesting twins. They arise from a single egg, as is shown by their size and by the history of certain types of cleavage destined to produce these doublets. Whether they owe their origin directly to environmental effects, i.e., whether they appear more often under certain exceptional conditions in the environment, remains somewhat uncertain. Penners reports that he has obtained them when the egg-capsules have been brought from a low temperature (10 degrees C.), where entirely normal development occurs, to a higher temperature (15 degrees to 20 degrees), and also when the water was lacking in oxygen. Five cases in all are reported in which something is known of their early history. Case I (10–12 degrees C. to room temperature). An egg was found with two large blastomeres filled with yolk, and three micromeres (Fig. 172*a*). The two large cells are interpreted as each equivalent to the D-quadrant of a normal embryo. In later development, a bladder-like protrusion appeared in the middle of the micromere field (Fig. 172*b*), that later collapsed. A Janus embryo developed (Fig. 172*c*) from this egg. Case II (20 degrees C.). An egg was found divided into two large and two small cells. The two large cells

contained yolk, and are called D-cells, D (II) and D (I). The smaller cells are called micromeres; a condition similar to that in Fig. 172*a*. This egg also gave rise to a Janus embryo. Case III (14–15 degrees to 25 degrees C.). An egg that had divided into two equal cells gave a typical Janus embryo. Case IV. The

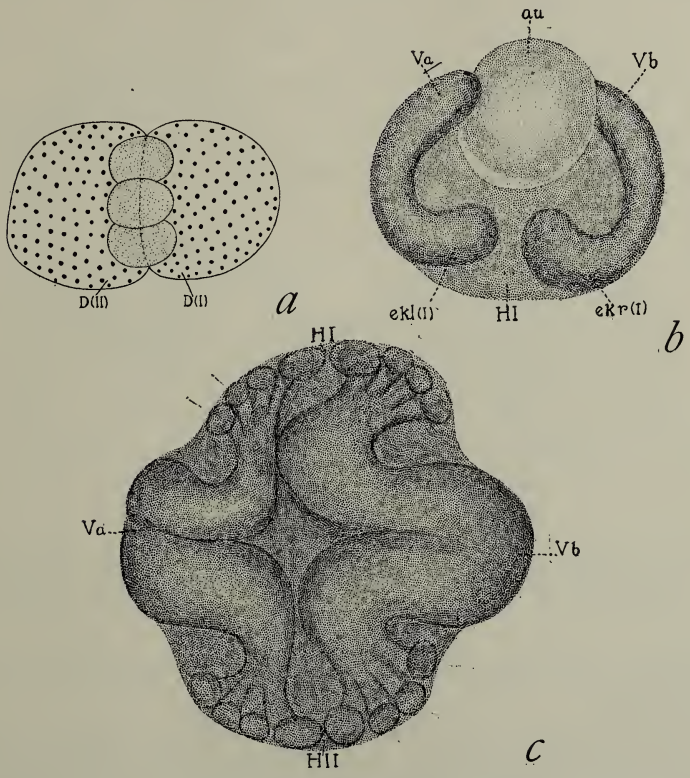


FIG. 172.—Janus-type of development of egg of Tubifex. *a*, two large blastomeres and three micromeres are present; *b*, later stage of same as seen from the side (there is a bladder-like protrusion in the micromere field); *c*, the Janus-embryo that developed from this egg (the two single heads are turned in opposite directions, and from each head germ-bands extend right and left, that later combine to form single trunk regions on each side.) (After Penners.)

AB blastomere of a normal egg was treated with ultra-violet rays. The CD-cell continued to develop at a higher temperature (14 degrees C.). The AB-cell had not been killed, but its further development was delayed. Later the larger CD-cell was found to have divided into two equal cells D (I) and D (II) (Fig.

173*a*, *b*). Two first somatoblasts, 2d (I) and 2d (II) were also present. The condition of this egg in later stages is shown in Fig. 174*a*, *b*. On each side of the egg two mesoderm cells, Ml and Mr, are present, as well as two cells representing the ectodermal bands (Nl, Nr). A somewhat abnormal Janus embryo developed from this egg. The doublet, after 53 days, had formed a twin worm (Fig. 174*c*). It had bifurcated anterior and posterior ends, but a common trunk. The half nerve cords crossed at the tail bifurcation. Case V (from 10 to 30 degrees C.). A 2-cell stage was kept for three hours at a higher temperature, then returned to the cold. Next day the larger cell, CD, had divided equally into two parts, as had AB also. Later both D-cells

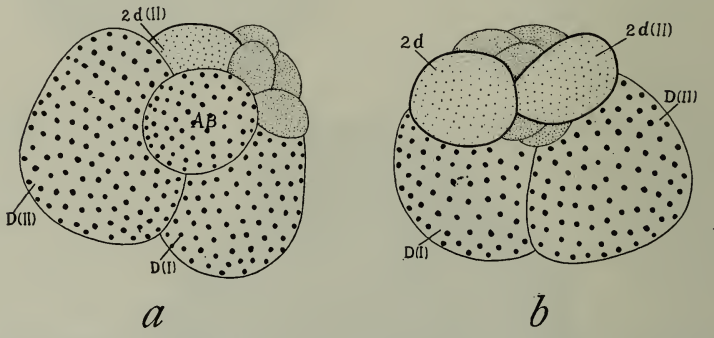


Fig. 173.—Two views of an egg in which the AB-cell had been injured by ultra-violet rays. Later the larger CD-cell divided into two equal cells D(I) and D(II). (After Penners.)

produced a first somatoblast cell. It was then killed for further examination.

Penners thinks that when the first division is equal, each of the two cells contains parts of each pole-plasm. The subsequent whole development of each half, he attributes to the presence of these materials in each blastomere. In support of this view he gives a section of an egg that had divided into two equal cells. There were two micromeres also present, but these are outside the plane of this section. In each large cell (DI, DII) there was a pole-plasm area and an antipole-plasm area. These were so clearly marked that there could be no doubt as to their presence. It seems to follow that the original pole-plasms had been divided by the first cleavage plane, or else two pole-plasms had been present in the original egg and the division plane had passed

between them. Penners adopts the former view, which seems the more probable interpretation in connection with other evidence that he gives, especially in the case where the CD-cell was induced to divide equally at a higher temperature. Nevertheless, the possibility of the other interpretation need not be entirely disregarded —at least such a condition should first be looked for. The

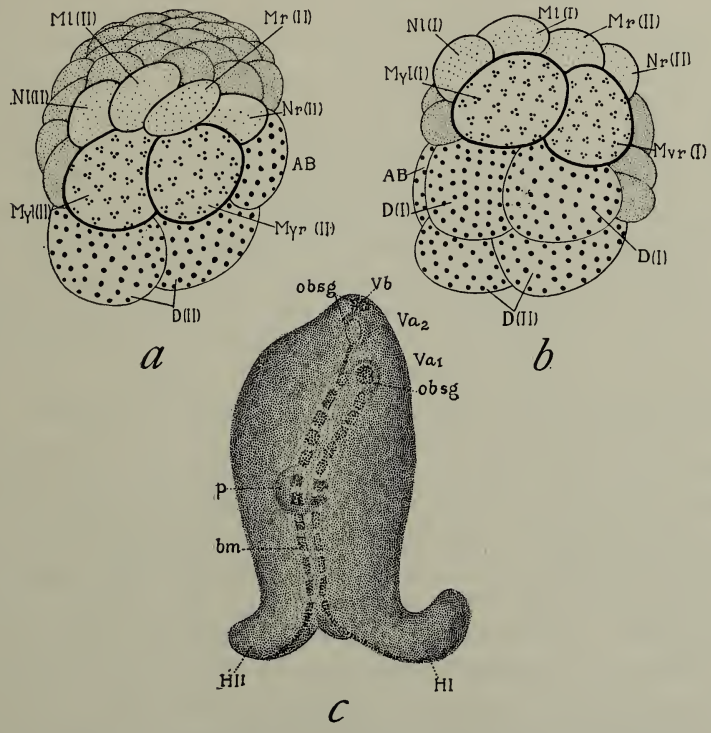


Fig. 174.—*a* and *b*, two side-views of later stage of egg drawn in Fig. 173; *c*, later embryo that developed from same. (After Penners.)

occurrence of eggs with two diploid nuclei—eggs that have arisen by an early fusion of two cells, has been reported, both from direct evidence (Doncaster '14) and from indirect or genetic evidence (Drosophila), but whether the presence of two nuclei would cause two polar fields to arise is of course quite unknown at present. It is also possible that two such fields might develop rarely in an egg with a single nucleus. However this may be, the point at issue remains the same, namely; that the occurrence of the

polar materials in two D-cells leads each of them to develop in the same way as does the D-quadrant of a normal embryo, and consequently to give rise to two more or less independent embryos. The condition of duplicitas cruciata is a secondary product of their union; for as the divergent arms of the ecto-mesodermal bands extend around the egg, the right side of one and the left of the other half approximate, as do the two halves of the same bands of the normal embryo. Hence, while each half has a single head and body, the tail ends are made up of parts of each embryo. The median axis of the head lies, therefore, at right angles to the median axes of the tails, hence the name duplicitas cruciata.

If Penners' evidence is accepted as sufficient,—as it seems to be in the main—the question of interpretation still remains. He lays all the emphasis on the presence of the two pole-plasm-materials in each blastomere, but accepting this as a fact his conclusion leaves out of account the nature of the division itself. We know too little at present about the kind of processes taking place in these determinative types of cleavage, in which the end-products of the division have become highly specified, to eliminate entirely the possibility that something is then taking place (or has taken place preparatory to these divisions) that is the essential factor in the end-result. In other words: the process of equal division of the egg (or of the D-cell of the 2-cell stage), rather than the concomitant equal division of the pole-plasms may be such that it differs from the unequal division of the whole egg in just those features that make the latter, but not the former, determinative. Until this question can be settled in other ways, the results from Tubifex do not seem to me to be as conclusive as Penners believes them to be. By way of illustration, I should like to recall certain features of the spiral type of cleavage shown by these and other eggs with determinative cleavage. Here, alternately, the egg divides by dexiotropic and leiotropic cleavages that are prepared for in each cell before the cleavage takes place. These divisions, including the formation of the 2d cell, are internal and specific, and preparatory to the subsequent differentiation of each kind of cell. Moreover, the sequence of the divisions is so definite that predictable regions of the egg pass into definite cells. Should there be present at the time, colored granules or otherwise visibly different materials, it might seem that they determine either the form of the cleavage, or the subsequent dif-

ferentiation of the cells, but it might be quite otherwise. The division itself might contain the essential features that affect the fate of the cells, and the granular or visible regional differences of the egg be no more than symptoms of these changes in the sense that their presence in certain regions necessitates their occurrence in certain blastomeres as a result of a definite relation between the orderly sequences of the division. The alternate changes from right to left and then from left to right, etc., that are characteristic of these divisions can hardly be due to the presence of corresponding regional substances already laid down in the egg. This possibility is excluded when, as the evidence shows, the first cleavage may be in any meridian of the unsegmented egg. The mosaic character of the cleavage with its accompanying localizations of differentiating factors, is something given, but not preformed, in the unsegmented egg. The mosaic cleavage from this point of view is largely an epigenetic phenomenon. As Wilson has aptly expressed it, the cleavage pattern is predetermined, but not prelocalized.

Injury to One Blastomere of the Egg of Cyclops

In several entomostracan crustacea whose cell-lineage has been worked out, the germ-cells, endoderm and mesoderm, are set aside at a very early stage. For example, the normal development of Cyclops viridis has been studied by Fuchs ('14) and by Maria Jacobs ('25) and experiments made on the eggs. The eggs, when set free in two strings, remain attached to the female. They assume various shapes as a result of mutual pressure in the jelly capsule. The first cleavage always cuts through the shortest diameter of the egg, but not necessarily through the pole of the egg. The two ends of the first segmentation spindle differ from each other in that there are numerous cytoplasmic granules around one pole. The blastomere that comes to contain the granules, gives rise to part of the ectoderm and mesoderm and also to the germ-cells and the endoderm. The sister blastomere, the one lacking granules, gives rise to the rest of the ectoderm and mesoderm. The second cleavage is at right angles to the first plane of cleavage. In one of the two cells the granules are still visible at the poles of the spindle (Fig. 175*a*), but are more numerous at one than at the other pole. The third and fourth cleavages

are also equal. At the 32-cell stage, two cells (Fig. 175*d*; *Kz*, *Enz*), the primitive germ-cell and the primitive endoderm-cell, shift their position. All the other cells, except these two, next divide giving rise to 62 cells (Fig. 175*e*, *f*). At the next cleavage stage the primitive germ-cell moves more into the interior, and there divides into two cells; the two primitive endoderm cells

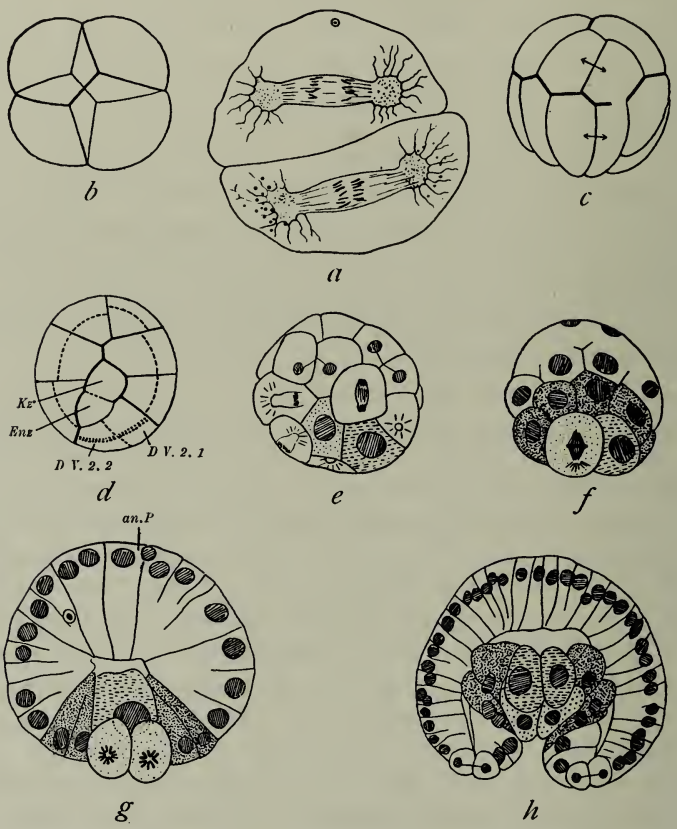


Fig. 175.—Normal cleavage and gastrulation of the egg of Cyclops. (After Jacobs.)

protrude, during their division, from the surface of the mass of cells (Fig. 175*g*). At the ninth division gastrulation occurs (Fig. 175*h*); a cell-plate consisting of the two primitive germ-cells and four endoderm cells and 24 to 32 mesoderm cells sinks into the interior. The lips of the blastopore come together and close.

It appears from the foregoing account that the cleavage axis, that later becomes the embryonic axis, is determined by the accidental shape of the egg resulting from the mutual pressure of the eggs. The embryonic axis bears, therefore, no constant relation to the polar axis as determined by the attachment of the ovarian egg, or to the location of the polar bodies. Nevertheless, the method of distribution of the granules during the first and second cleavage indicates that differentiation is beginning in the distribution of material and that it follows the cleavage. The localization of the median plane in relation to the cleavage plane is somewhat uncertain, but may be first indicated by the shift in position of the early germ-cell and endoderm cell. From a study of eggs, isolated before the polar bodies are extruded and from a study of eggs of particular shapes, Miss Jacobs has shown that the maturation pole bears no constant relation to the cleavage, but gastrulation takes place at the meeting point of the first two cleavages, and that the first cleavage plane coincides with the median plane of the embryo.

One of the first two blastomeres can be injured by exposing it for one and a half minutes to ultra-violet light. When the distance of the point of injury is so adjusted that only one blastomere is affected, its subsequent division may be suppressed. The other blastomere continues to develop while remaining still in contact with, and compressed against, the other blastomere. It divides as in the normal egg.

Two kinds of results are expected and two are observed, according to which of the first two blastomeres is injured. In subsequent stages one blastomere gives rise to germ-cells and endoderm, the other does not produce these cells, but both produce ectoderm and mesoderm.

In later stages the ectoderm of the uninjured half extends over the material of the injured half, partly enclosing it.

By means of sections it was found that when the two nuclei of the primitive germ-cells were present in the developing half, there were indications that endoderm cells were also present in this half-embryo. Endoderm was absent in those half-embryos that did not contain germ-cell nuclei. In both kinds of embryos mesoderm cells were present, and, of course, also ectoderm. Half-embryos without germ-cells invaginate fewer cells (presumably mesoderm) than do those with germ-cells. The number of cells in

the half-embryos was half that of the normal embryo, and the cells in both were the same size. In the nauplius stage, when legs appear, the half-embryos should be distinguishable from whole embryos. It appears that half-structures were present in both kinds of embryos, but only young stages were examined, and, owing to irregularities and abnormalities the half-structure was not strikingly shown.

Eggs were also examined in which one of the first four, or two of the first four, or three of the first four cells were injured. The results are consistent with the foregoing, but do not add any further facts of importance. The evidence shows clearly that ½ the ¼ blastomeres of Cyclops viridis develop in contact with the injured half as a partial embryo; but it is probable that the presence of the injured blastomere has practically no influence on the result.

Injury to the Egg of Insects

The eggs of insects differ in their early stages of development so greatly from the eggs of the types thus far described, that injury to the egg presents an entirely new set of problems. Hegner ('09, '10, '11) made many experiments of this sort, injuring, by means of a hot needle, the posterior end of the egg of the beetle, Calligrapha. These experiments were made in order to find out whether the cytoplasmic materials at the posterior end of the egg from which germ-cells are derived, or more specifically whether the granules contained in that part of the egg, determine the future course of the cell or cells in which they come to be contained. Hegner found, in fact, that when the posterior end is injured the germ-track does not develop. It does not follow, however, that the granules in the posterior region are "organ forming," but at most that from this region of the egg the germ-track develops, and in its absence (due to injury) the other parts of the egg do not make good the loss of this part, for, as other experiments made by Hegner and later by Reith ('25) have shown, the injury to any part of the egg leads to a failure in the development of those organs that normally develop from that part. Nevertheless it might still be true that it is the granules in this region that are the "germ-cell determinants." Hegner attempted to settle this point by centrifuging the eggs, but the results are not convincing. The earlier experiments that

Hegner ('08) had made by pricking the posterior end of the egg, causing some of its material to flow out, also do not give crucial data in so far as the granules are concerned, but do show that in the absence of this region the germ-cells do not appear in the later embryo.

In addition to the experiments relating to the germ-cells,

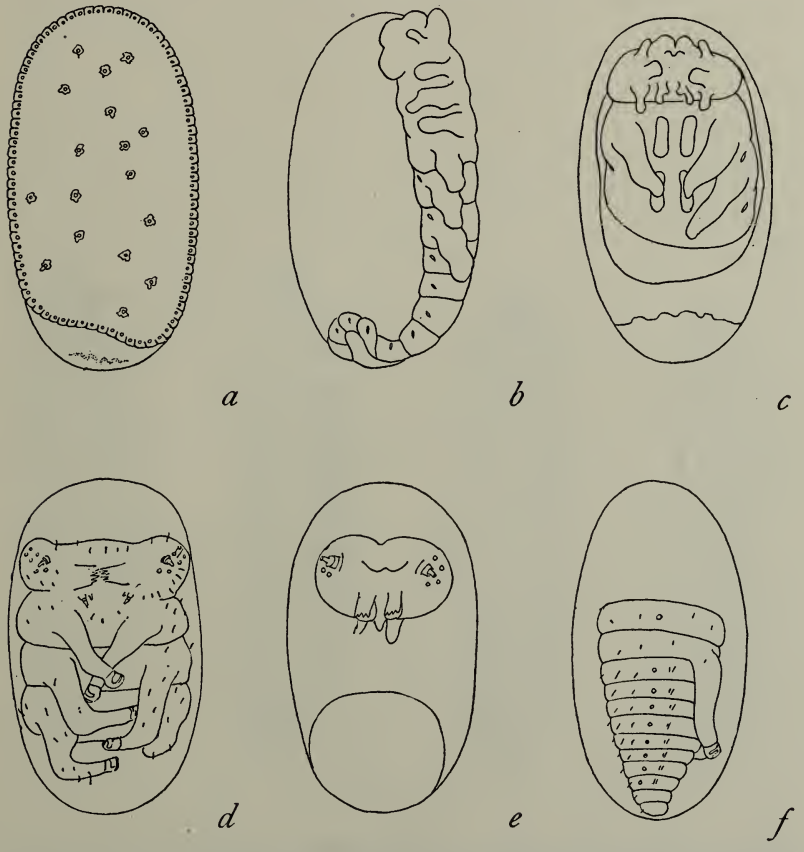


FIG. 176.—Parts of embryos that developed after injuring the eggs of Calligrapha with a hot needle. (After Hegner.)

Hegner examined the results of injury to different parts of the egg and embryo. When the anterior or posterior region of the egg is injured before cleavage, there are corresponding defects in the embryo (Fig. 176). A rather striking result was obtained when the anterior or posterior region of a later stage was injured

at a time when the embryonic organs had begun to develop. The uninjured part then continued to develop independently of the injured part whose progress had been stopped.

Similar experiments have later been carried out by Reith ('25) on the eggs of the house fly (Musca domestica). The newly laid egg of the house fly was injured by means of a hot electric needle that was touched to one end (or the other) or to the middle region of the egg. At the time of injury the segmentation nuclei in the interior of the egg had not reached the surface. There may be one, or two to eight of these nuclei present. They are, as a rule, beyond reach of the heat and remain uninjured, but the surface layer of the egg and the underlying parts are killed and coagulated by the heat. These injured parts remain a component part of the rest of the egg, but fail later to become cellulated. When the anterior end is cauterized, the head end of the embryo fails to develop (Fig. 177*b*) while the remaining portions of the egg form the same structures as they would normally produce (Fig. 177*e, f*). When the posterior end is injured (Fig. 177*c, d*) the rest of the embryo develops (Fig. 177*g, h*) and produces those parts that come from the same regions in the normal egg. When the middle region is injured the anterior and posterior organs develop.

Serial sections of the early stages of these cauterized eggs show that when the central nuclei wander to the surface to form there the blastoderm, those that come in contact with the coagulated cytoplasm fail to penetrate it, but accumulate at its edge where they swell up and stop dividing (Fig. 177*e-h*). The injured portion does not become overgrown by the ectoderm of the uninjured part. In later stages, the edge of the ectoderm seems to turn in to cover the end of the embryo that is in contact with the injured portion, thereby cutting the latter off from the embryo. But there is no evidence that any regeneration of the missing regions takes place. The organs that develop appear to have the same size as the same organs in the normal embryo (Fig. 177*e-h*). There is no compensating regulation of the developing part.

The results show that in this egg, and presumably in those of other insects with centrolecithal cleavage, the embryo is foreshadowed in the cytoplasm that covers the surface of the egg into which the segmentation nuclei migrate. The different cyto-

plasmic regions are as determinative as though they were divided up into cells at each consecutive division as, in fact, they are later. The evidence from this source is consistent with that from a study

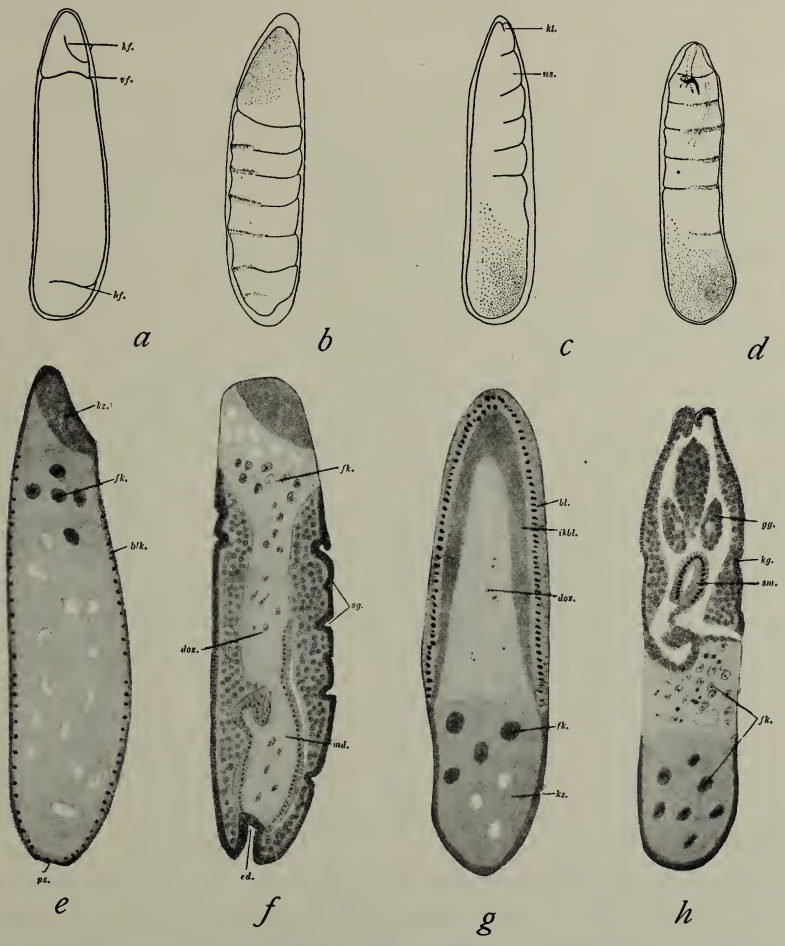


FIG. 177.—Parts of the egg of Musca killed by heat. *a*, normal embryo; *b*, anterior end injured; *c* and *d*, posterior end injured; *e* and *f*, sections through egg injured at anterior end as in *b*; *g* and *h*, sections through egg injured at posterior end as in *c*. (After Reith.)

of mosaics and gynandromorphs of Drosophila. In the latter, however, the nuclei themselves have become different, owing to irregularity in the early cleavage stages; they carry these differences with them into the cytoplasmic areas where their pecu-

liarities come to light. The information gained from these two sources is mutually illuminating in showing that even in such eggs where the cytoplasm plays an important rôle in determining the regional differentiation of the embryo the character of the nuclei is also determinative for those characters that depend on their constitution.

A quite different picture of the localizing factors in the insect's egg is given in the recent experiments of Seidel ('26), who studied the effects of tying off posterior portions of the egg of a dragonfly, Platycnemis pennipes, as well as of injuring these regions with an electric needle. The egg shortly after its deposition and while the cleavage nuclei are dividing (but before these have reached the surface) was constricted in different regions by tying a hair around the egg. The egg was separated into two parts. If the constriction lay in the middle of the presumptive embryonic region, i.e., just behind the middle of the egg (Fig. 178*a*) a blastoderm developed in the posterior region; but the region anterior to the constriction did not form a blastoderm. There was no head formed in this region. If the constriction was not complete, however, a head developed (Fig. 178*b*). A histological examination of the anterior region showed that it was not dead. The yolk-cleavage had taken place, but at the surface of this part a plasma surface layer only was present. A blastoderm had not developed. On the other hand, when the constriction cut off only the posterior tip of the egg (Fig. 178c), the development of the blastoderm was not prevented in front of the constriction and a normal embryo was formed (Fig. 178*d*). But if the constriction lay a little further forward (Fig. 178*e*) no blastoderm appeared either in front of, or behind the constriction (Fig. 178*f*). The subdivision of the yolk into cells and the formation of a plasma layer went on. These results seem to show that the development of the blastoderm is contingent on the presence of a region near the posterior end of the egg. The region does not extend, however, to the most posterior region of the egg. For more precise determination of this initiating region, a more refined method was needed. By means of a hot cautery-needle, the location of the injury could be more accurately controlled. An hour after burning, a sharp line between the dead and the living parts became visible (Fig. 178*g*, *h*, *i*). The results were the same as in the constriction experiments. The egg is about 36–40 units long.

Injury of the posterior end extending over 3 or 4 units does not prevent the formation of an embryo in the anterior region. The limit varied somewhat in different cases between 2 and 6 units.

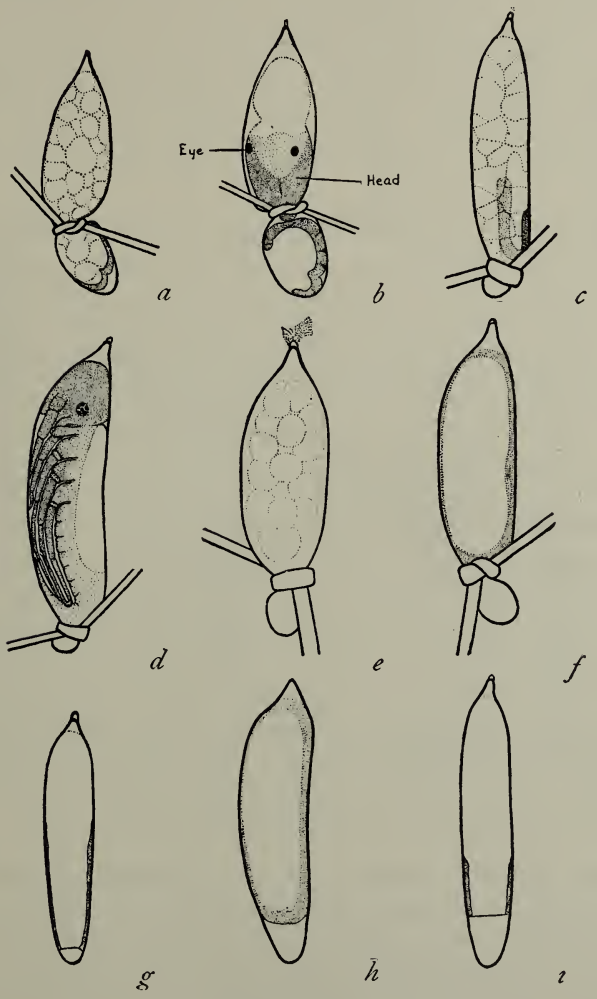


Fig. 178.—*a–f*, eggs of dragon-fly that have been constricted by hair at the posterior end; *g–i*, eggs whose posterior ends have been killed by heat. (After Seidel.)

When the posterior end was killed anterior to these limits, the blastoderm did not develop in the anterior part. Seidel concludes that there is a posterior zone whose presence is necessary for

the development of the embryo. He calls this region the determination zone.

The preceding experiments were made on eggs during the early cleavage stages. Further experiments were undertaken to find out whether this dependence lasted into later stages: that is, whether having initiated the development anteriorly the initiating zone could then be removed without stopping the progress of development forward. The results showed that 8 to 14 hours (at 22.5 degrees C.), before the blastoderm becomes visible, the

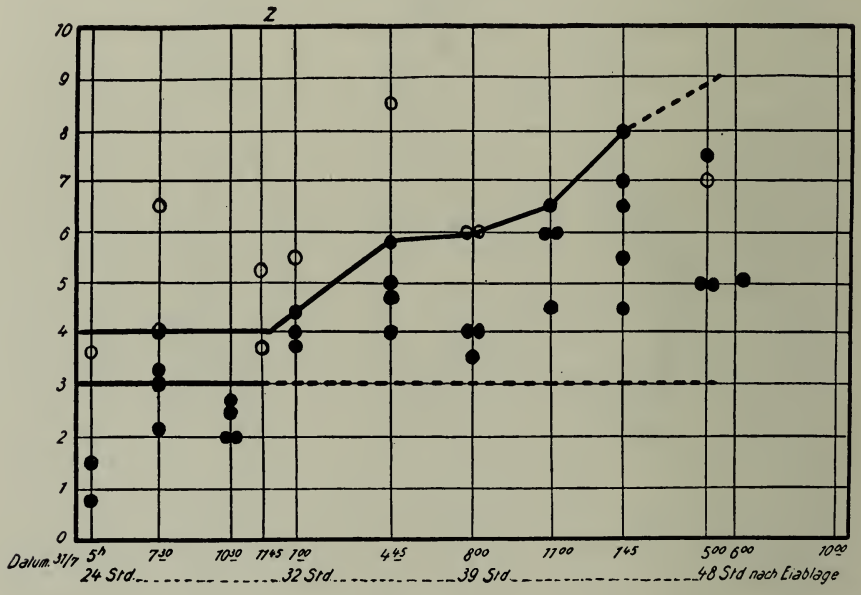


Diagram V

dependence on the presence of the posterior initiating zone (center) has ceased, and that this zone has moved forward.

Experiments were undertaken to discover whether there is a progressive forward influence beginning in the initiating zone that is correlated with the age of the embryo, i.e., of the stage already reached. If, for instance, in the earlier stages an injury of 5 units prevents development, and if it were found in a later stage that the injury must extend further forward to 7 units to produce this effect, the result would indicate a progressive forward movement of the initiating influence. Between 24 to 48 hours

after deposition of the eggs, the eggs were burnt at intervals of every three hours. The results are given in Diagram V. The abscissae indicate the time of burning, the ordinates the length of the injured posterior end (1 unit to 24 units). Each experiment is indicated by a circle; the black circles mean that a blastoderm developed, the open circle that no blastoderm appeared. The diagram shows that, up to 31 hours after deposition, extension forward of the zone has not taken place, since injury of more than 3 to 4 units gives no development. The picture then changes, for, with the advance in development the dependence shifts forward. At 32 hours and afterwards there is evidence of an advance, since longer regions if progressively injured do not interfere with anterior development. At 32 hours the limit is 5.5 units; at 35¾ hours the limit is 5.8 units; at 39 hours the limit is 6 units; at 42 hours it is still greater. With advancing injury smaller and smaller pieces of the thorax developed until finally only the head was left. The results show that there is a progressive forward influence of some sort taking place. How this initiating impulse (?) from the more posterior part of the egg produces its effect is not at all evident. Its influence is observed before any visible development of the blastoderm occurs. There is no material present in the posterior region that moves forward in normal development, hence the absence of the impulse can not be due to an inhibition of the movement of materials forward. In fact, the first appearance of the blastoderm on the surface of the egg is near the middle of the egg, i.e., about 10–18 units from the posterior end; the "center" therefore is neither a budding zone, nor differentiation center. Seidel gives it the vague name of a determinating center, without attempting to state how it works.

CHAPTER XIX

THE FATE OF CELLS AND THEIR LOCATION

The "isolation experiments" have shown that in eggs with so-called determinative changes, the approximate fate of some of the blastomeres is rather narrowly fixed at the close of specific cleavages. The experiments on the insect's egg, in which certain regions of the egg are injured by a hot needle, have also shown that cytoplasmic regions of these eggs are early set aside to produce definite parts of the embryo. Nevertheless, in both cases, some of the cells, at least, such as the ectodermal and endodermal cells, appear to have retained rather wide powers of adjustment which are comparable to a similar latitude shown by all or most of the early formed cells of those types having a so-called indeterminate cleavage. It is these "powers" of readjustment that have often been singled out as the most significant features of development, but however significant they may appear in a theoretical sense, the processes by which this is accomplished are still unknown. The evidence indicates also that after cleavage has taken place, the possibility of readjustment is more limited, and is more confined to readjustments within regions that have already been determined as primary organ-systems. Driesch's experiment ('96), in which the inner end of the invaginating archenteron of the sea-urchin was cut off, was one of the first attempts to determine the possibility of such readjustments within a secondary organ-system. The results showed that, after the removal of the inner end of the early archenteron, the remaining portion of the archenteric tube still produced the same parts as does the whole archenteron. In other words the archenteron at first showed a power of readjustment similar to that of the isolated blastomeres of the sea-urchin.

In recent years other evidence bearing on the prospective fate of different regions of the blastula and gastrula stages of amphibia has been obtained by grafting experiments. A series of experiments by Spemann ('18) demonstrated that the ectoderm of the

polar hemisphere of the early gastrula stage of Triton may become either surface ectoderm or neural plate. The polar hemisphere of an early gastrula stage was cut off from the other half. This polar ("animal") hemisphere was then rotated through 180 degrees and re-implanted on the other ("vegetative" or gastrula) half. The two reunited, and later a normal neural plate appeared that extended from the "lower" into the "upper" hemisphere. This material, out of which the anterior end of the neural plate developed, would have normally become surface ectoderm. Conversely, the material that became ectoderm would have normally become part of the central nervous system, if left in place. Spemann ('18), carried out in another way some experiments that demonstrate the same potentialities of the ectoderm of the upper hemisphere. By means of a micropipette, a small piece of prospective ectoderm from an early gastrula was transplanted into the region of the prospective neural plate of another gastrula. The transplant became later a part of the neural plate of the host, and formed an integral part of its central nervous system. Conversely, a small piece of prospective neural plate from an early gastrula was transplanted into a region of prospective ectoderm. The transplant formed later an integral part of the surface ectoderm of the host. These experiments show that at the time of gastrulation, before the neural plate is laid down, the cells of the polar hemisphere have not yet had their fate determined (within the limits of the experiment), or if their differentiation has begun it may be reversed by a change to a new location. Other experiments to be discussed below confirm and extend this conclusion.

In the experiment just described, lighter and darker pigmented eggs of the same species (Triton taeniatus) were used. The difference in pigmentation made it possible to follow for some time the transplant and determine accurately its fate. Similar experiments have also been made by Spemann ('21) in which the lighter-colored gastrula of Triton cristatus was used as one member of the pair, and the darker-colored gastrula of Triton taeniatus as the other member. The eggs and young stages of T. cristatus are without pigment, and white, or slightly greenish. The eggs and young stages of T. taeniatus show all degrees of pigmentation from yellow to brown or even black. A small piece of the pale ectoderm of T. cristatus from a young gastrula stage was trans-

planted into the region of the prospective neural plate of a corresponding stage of T. taeniatus (Fig. 179*a*). When the neural

FIG. 179.—*a*, an early gastrula stage of Triton tæniatus in which a small piece of prospective ectoderm of T. cristatus has been inserted in the region of the prospective neural plate of this embryo; *b*, later stage of *a*; *c*, a cross-section through the embryo that developed from *a* and *b*. The right side of the neural tube and the right eye vesicle have come from the implanted piece. *d*, an early gastrula of T. cristatus in which a piece of prospective neural plate material of T. tæniatus has been inserted in the prospective ectoderm of this embryo. In later stages, *e* and *f*, the implant has become part of the surface ectoderm. (After Spemann.)

plate of the host appeared (Fig. 179*b*) the transplant was found at the anterior end of the neural plate. When the plate rolled

in to form the neural tube, the transplant formed part of the wall of one side (Fig. 179c). The eye-vesicle of this side had been given off by the transplanted cells as seen by its pale color. It was somewhat less developed than the other eye of the host on the opposite side.

The reciprocal combination was also made. The piece removed from the T. taeniatus gastrula (to make room for the transplant) was inserted into the region of prospective ectoderm of a gastrula of cristatus (Fig. 179*d*). Later, when the embryo developed, a black region of ectoderm at the side of the neck was present (Figs. 179*e*, 179*f*). From this region the gill-slits and gills developed, whose surface layer of ectoderm had come from the prospective neural plate of the other species, but the endoderm and mesodermal part of the gill-slits belonged to the host.

These results show not only that the future of certain ectodermal regions of the gastrula is determined by their position, but that the same relations hold good when the transplant belongs to one species and the host to another. The reaction is a common property of both embryos.

Spemann had suggested in 1903 from his constriction experiments, in which double-headed embryos were produced, that the form and size of the neural plate is in some way connected with the presence of the archenteron; and later ('18) and in collaboration with Hilde Mangold ('24) he was led to the view that the presence of endo-mesoderm beneath the surface of the ectoderm is the immediate cause of the formation of the neural plate. The proof of this was first found by Marx ('25), who removed a small piece of the roof of the archenteron (of a T. cristatus gastrula) and implanted it in the blastocoel cavity of a young gastrula (of T. taeniatus). The inserted piece called forth the development of a neural plate and tube. Geinitz ('25) has carried out numerous experiments of this kind between several species and genera of amphibians, and obtained similar results. For example; the ectoderm of a Bombinator embryo anterior to the blastopore (the future neural plate) was lifted off and removed, and a piece of the underlying archenteric roof (at the time composed of future chorda-mesoderm) was taken (Fig. 180*a*) and transplanted into the blastocoel of a young taeniatus gastrula. The result is shown in Fig. 180*b*. The neural tube of the main embryo (recipient) is present (at the left) and the induced neural

tube (from the donor) to the right. The two diverge in front and converge behind. Similar results were obtained between Rana temporaria (donor) and Triton taeniatus (host), and Rana esculenta (donor) and Triton alpestris, Bombinator and Triton taeniatus.

More exact data relating to the potential power of regions anterior to the dorsal lip of a young gastrula to induce the formation of a neural plate have been obtained by Bautzmann ('26). The piece of the donor was inserted into the blastula cavity

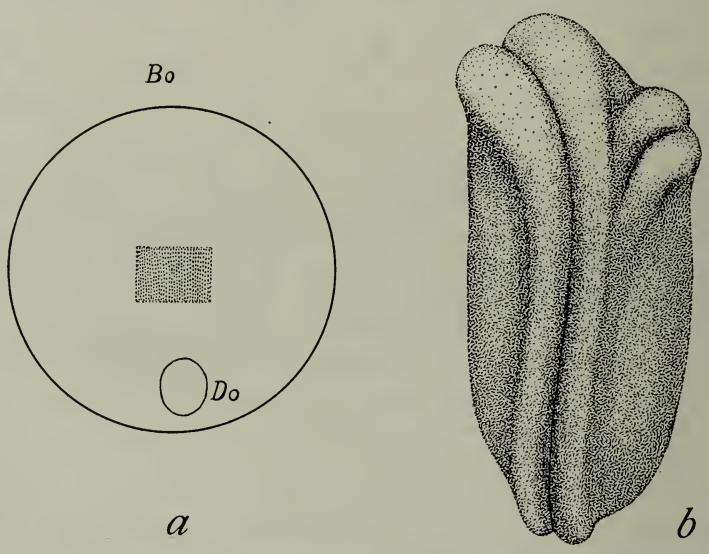


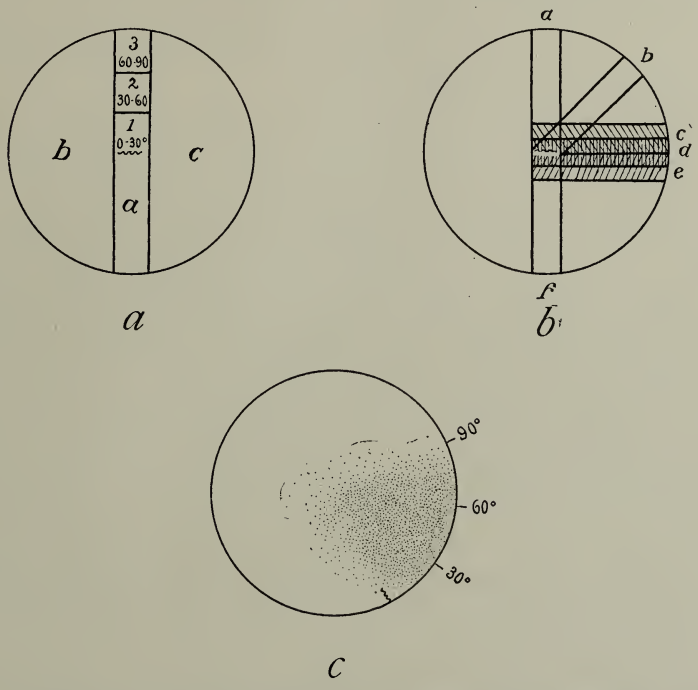
FIG. 180.—*a*, diagram of gastrula of Triton. A piece of the surface ectoderm of the neural plate of this neurula had been removed and a piece of the dorsal wall of the archenteron taken out and inserted in the blastula cavity of a young gastrula. The embryo that developed from this gastrula and its transplant is shown in *b*. A small secondary neural tube is present at the side of the anterior end of the primary neural tube. (After Geinitz.)

of a younger embryo (recipient). Pieces were taken at four different levels (0–30 degrees, 30–60 degrees, 60–80 degrees, 90–120 degrees, Fig. 181*a*), in front of the dorsal lip. The first three act as organizers, the last one does not, but produces a mesenchymatous mass of cells, or else is added to the primary somites. The anterior level of the chorda-mesoderm lies, therefore, about 90 degrees in front of the dorsal lip. This corresponds approximately with Vogt's results (see below).

Other pieces were removed in an oblique upward direction

(Fig. 181*b* along *b*). The first and second pieces acted as organizers, the third failed, the fourth induced some buckling of the surface, but no definite neural plate. Two series were taken across and a little above the blastopore (Fig. 181*b*, along *c*, *d*). The more proximate pieces became activators.

Another series of pieces was taken across and below the level



a b c

FIG. 181.—*a*, diagram showing the levels, in degrees, from which pieces were successively removed in front of the dorsal lip of a gastrula of Triton; *b*, diagram showing where other regions were tested; *c*, diagram showing the region of mes-endoderm at the beginning of gastrulation. (After Bautzmann.)

of the dorsal lip (Fig. 181*b*, along *e*). The first piece *behind* the dorsal lip did not give induction. This material (yolk) behind the dorsal lip does not contribute normally to the chorda-mesoderm. The next piece produced an excrescence which, on sectioning, was found to consist of mesoderm inside, and a thickness of ectoderm on the surface. The third and fourth pieces gave similar, but inconclusive results.

Pieces taken from the region behind the blastopore (Fig.

181*b*, along *f*) in the yolk plug gave no effect. The results of these experiments go to show that the organizing materials extend forwards about 90 degrees in front of the point at which the dorsal lip first appears and also extend laterally to the extent shown by the pigmented area in Fig. 181*c*. This region corresponds approximately with that of the gray crescent of the frog, and Vogt and Goerttler and Banto have identified a specially pigmented region of the egg of the Axolotl with the gray crescent of the frog. Even in Triton alpestris and taeniatus there appear to be indications of a similar crescent.

It had been shown by Spemann and Mangold ('24) that a piece of the dorsal lip of one species of Triton may act as activator in the embryo of another species of Triton. Geinitz ('25) has shown that this property is possessed by a much wider range of species. The following combinations were tried out: three species of Triton (taeniatus, alpestris, cristatus), Pleurodeles waltli, Amblystoma mexicanum, Rana temporaria, Rana esculenta, Bombinator pachypus. Pieces of chorda-mesoderm were inserted into the blastula cavity of a younger stage (blastula, or young gastrula). The induced embryo has the same characteristics as those shown in autoplastic grafts of the same kind. In most of the experiments the recipient was one of the urodeles; the anurans are not so well suited to play the rôle of recipient, and did not give favorable results. The experiments, especially those in which Triton was the recipient and Bombinator the donor, show clearly that the reaction is one of a general kind, and not confined to the cells of each species.

An extensive series of experiments testing the possibilities of the ectoderm from the polar region of the blastula and young gastrula stages of Triton, has been carried out by Otto Mangold ('24). By means of a small pipette, a piece of the pale ectoderm of Triton cristatus was removed and inserted into the region just behind the dorsal lip of a young gastrula of Triton alpestris (Fig. 182*f*). The piece becomes quickly incorporated in the host as a small oval disk, and is subsequently carried into the gastrula-mouth. Owing to the paler color of the graft its presence inside the embryo can sometimes be seen through the walls of the later embryo. Out of 29 such implants 20 were later found in the mesoderm, and 9 as rounded masses lying on the floor of the archenteron.

When the implant is placed on the border of the yolk-field (behind the blastopore where the lateral lip is expected to appear later) the lateral lip may appear outside of or inside of the graft. The implant is here also carried into the interior and incorporated in the chorda-mesoderm.

When the implant is placed in the dorsal lip itself, or in a lateral lip, it remains generally in place and interferes with the normal invagination. Spina bifida embryos result, but occasionally the implant passes into the mesoderm.

The outcome of these experiments may be summarized as follows: When prospective ectoderm from *the top of the egg of a blastula* or young gastrula stage of Triton is implanted at the lips of the blastopore, the graft is carried into the interior, and may become transformed into any one, or part of any one, of the following organs, e.g., notochord, mesoblastic somites, pronephros, splanchnopleure (Figs. 182*a-e*).

Ectoderm taken from a *gastrula with closed blastopore*, i.e., ectoderm destined in the normal course of events to become the outer covering of the embryo, produces, if carried into the mesoderm, mesodermal somites; if carried into the endoderm it may become incorporated in the roof or sides of the archenteron. It follows that even after the close of the blastopore the potency of the ectoderm is still not limited to the formation of ectodermal tissues.

Ectoderm taken from the neural plate of an embryo with closing neural tube and implanted into a younger gastrula will, if carried into the interior, develop into a piece of the brain or into an eye vesicle. If ectoderm that is already determined in a neurula stage is carried into the mesoderm (after transplantation) it will not develop into mesoblastic somites, but remains as a foreign body that may become attached to the overlying ectoderm.

It is not essential for the formation of meso- and endodermal organs that these materials should take the normal course to reach their destination, i.e., that they should be invaginated at the blastopore rim. If they reach by abnormal paths a final position in the mesodermal sheets, they become incorporated in the organs that normally develop from these layers.

If there should be a local excess of material in the mesoderm resulting from the implanted ectoderm, the elements of the

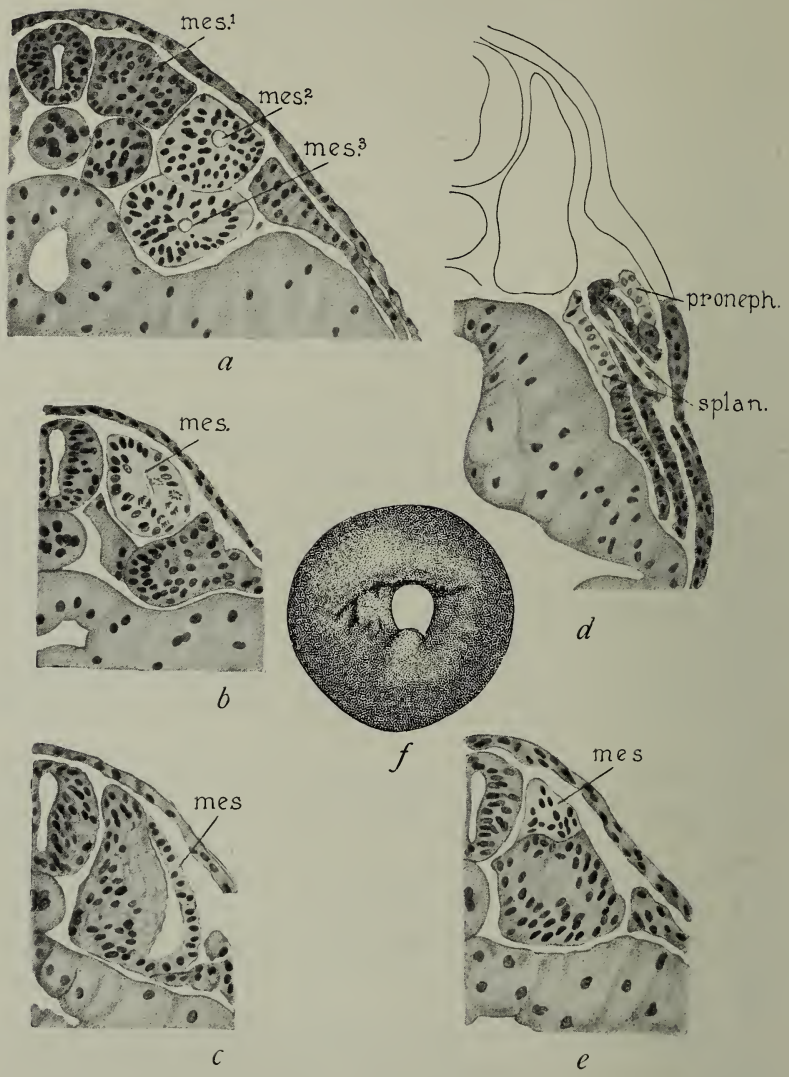


FIG. 182.—Small pieces of prospective ectoderm were grafted behind and to one side of the dorsal lip of the blastopore, as in *f*. Later, when the embryos had developed, sections were made, *a–e*, and the location of the implant determined by the difference in its color. In *a* it has formed a supernumerary somite; in *b* it has formed a somite in normal position; in *c* it has become the outer layer of a somite; in *d* it has become part of the pronephros; in *e* it has become part of a somite. (After Mangold.)

pronephros develop normally in size and form, but in larger numbers. Similiarly for the mesoblastic somites; normally sized somites are produced, while the remainder of the material forms accessory somites attached to the others.

These experiments of Mangold's, taken in connection with those of Spemann, go to show that the so-called ectodermal cells near the top of the upper hemisphere (more particularly those of the quadrant extending from the pole to the equator on the "ventral" side) may, if they are incorporated in the notochordal or mesodermal layer, take a part in whatsoever organ develops out of these layers. The probability is, therefore, that they would become typical functional cells of these organs. Spemann's experiment, in which the top of the blastula was rotated through 180 degrees, had also shown that the presumptive ectoderm may become the anterior end of the neural plate, hence nervous tissue. The exchange of pieces between prospective ectoderm and prospective neural plate cells has given the same result. It is apparent, then, that the fate of the early "ectodermal" cells is determined by their ultimate location in the embryo; in other words, they are undifferentiated at this time, and their subsequent differentiation is determined from without rather than from within.

If this were true for all the other regions, it would be difficult to see how differentiation could ever begin, but there is other evidence that points to the region in front of the dorsal lip of the blastopore as the center of the future differentiation of the embryo. It is this material which, when grafted elsewhere, sinks beneath the surface, and calls forth the development of the neural plate in the ectoderm. Presumably it acts in the same way in the normal embryo, when it is inturned at the lips of the blastopore, and becomes notochord and mesoderm. There can be little doubt, therefore, that this region is already different from the rest of the egg before the gastrula lip appears. The main question is, how long before? There is evidence from the cleavage of the frog's egg showing that from the 8-cell stage forward the side of the egg nearest to the gray crescent develops faster than any other region. This region corresponds to the future dorsal lip of the blastopore. It is probable that similar conditions hold for the egg of Triton. If this can be established it may be possible to trace back to the fertilized egg of the amphibian the organizing

center of the embryo around which the development takes place. Whether this region develops fastest, and takes the lead in the subsequent events, because there is present in it *after* fertilization (but not before) a more specific kind of "organizing" material; or whether it develops fastest because it comes to contain more of certain kinds of materials than do other parts (although all parts of the egg contain the same materials in different mixtures) remains for future work to establish. But even to assume that because a region develops faster than the rest of the embryo, it becomes therefore the organizing center (here the dorsal lip) is little more than begging the question at issue, because there is no other evidence to appeal to, which proves that more rapid development in one part than in another initiates the kind of change that would make it the organizer of such a structure as the dorsal lip of the blastopore.

Spemann found that when a piece of the anterior end of the neural plate of older embryos (neurula stage), in which the outlines of the plate are clearly present, is grafted into the ectoderm of another embryo, it is overgrown, and, sinking beneath the surface, differentiates into the same part it would have formed if left in situ. An eye, for instance, might result if the piece had come from the eye-forming region of the other embryo. On the other hand as has been stated above, pieces from the same region of an earlier embryo (gastrula), when transplanted to an ectodermal region, become ectoderm. It follows that at some stage between the early and late neurula stages the differentiation of the neural plate sets in. Spemann concluded that the critical point is reached when the blastopore is reduced to a horizontal slit-like line, i.e., near the time of its closure. From other evidence he concluded that the presence of endoderm beneath the ectoderm is probably the determinative agent that causes the differentiation of the ectoderm. Marx ('25), carried out later, in Spemann's laboratory, experiments to determine more exactly the time at which the change in the ectoderm takes place, and the way in which it is brought about.

When a piece is taken from the right anterior end of the region some distance in front of the blastopore of a gastrula of Triton taeniatus just before the neural plate is about to appear and transplanted to an embryo of T. cristatus in the lateral or ventral ectoderm, the implant sinks beneath the surface, and

its edges turn in to form a hollow vesicle resembling a part of the neural-tube. The result is the same whether or not a piece of the underlying chorda-mesoderm is carried over with the piece. This means, in the light of other evidence, that the differentiation of the plate at this time is irreversibly determined.

When a similar piece was taken from an embryo with a yolk-plug or slit-like blastopore (a stage younger than the last) the implant again forms a neural vesicle, provided a piece of the chorda-mesoderm is also carried along with the overlying neural plate material, but if the chorda-mesoderm is absent in the transplant, the transplant becomes a part of the surrounding surface ectoderm. When a piece is removed from a still younger embryo, one with a semi-circular blastopore, it becomes a part of the ectoderm into which it is transplanted.

It follows that the differentiation begins some time between the last two stages. The difference that was found in the second stage, in which the implant sometimes formed a neural vesicle and sometimes ectoderm, is due, according to Marx, to the presence of mesoderm in some of the implants and to its absence in others. He concludes that the differentiation of the piece is determined either before the piece is removed, or after it is removed (if it is not already affected) by the mesoderm. This is in accord with other results of his own and of Geinitz, showing that when a piece of the chorda-mesoderm is inserted into the blastula cavity it calls forth a neural plate in the overlying ectoderm.

A further study of the influence of the chorda-mesoderm on the development of the neural tube has been made by Lehmann by means of localized injuries in front of the dorsal and lateral lips of the blastopore of gastrula stages of Triton. In many cases the injury was not compensated for after the chorda-mesoderm had been turned inside. Defects were often found in the roof of the archenteron according to the location of the original injury. An injury in the earliest gastrula stage to the median dorsal lip brought about a defect in the fore-gut; while an injury to the same region of a later embryo caused defects in the notochord and mesoderm, but the neural plate above was unaffected. The results show that, in the beginning, the neural plate is little affected by the presence of defects beneath it, but in later stages of its development the effects become manifest. For example, when

defects are present in the chorda-mesoderm, the earliest stages of the neural plate take place normally up to the time of the lifting up of the folds and the beginning of their inrolling. The plate, when first formed, is sharply defined and shows no asymmetry. The explanation of this is found, Lehmann says, in the fact that even in the normal development, soon after the beginning of gastrulation, the neural plate is partly determined before it is underlaid by the roof of the archenteron. For its later elaboration, however, the presence of underlying chorda-mesoderm is needed, as shown in the later imperfect development of the plate when defects are present beneath it. In the presence of defects in the underlying layer the edges of the plate are poorly developed and, in comparison with normal regions, delayed in their progress. The defect is shown especially in the failure of the cells to assume the characteristic radial arrangement. It appears, then, from these results, as well as the earlier ones of Goerttler on Pleurodeles, that the roof of the archenteron is not essential for the first determination of the overlying plate, and that the region from which the neural plate will develop is self-determining at the time of gastrulation, or at least it is not dependent on chorda-mesoderm, but whether its independence arises from some sort of "determination stream" (Spemann, '18) emanating from the blastopore, or is self-determining at this stage cannot be stated. A somewhat similar conclusion has been reached in regard to the determination of the lens of the eye of amphibia where it has been shown that, in some cases at least, the lens may begin to form in the absence of the underlying optic vesicle, but its later development is dependent on the presence of the optic cup. It appears, then, from this work that the neural plate is partly self-determining, but also that it may be induced in other regions by influences arising outside of itself, namely, by the presence of chorda-mesoderm beneath.

CHAPTER XX

THE FUSION OF TWO EGGS TO PRODUCE ONE EMBRYO

Eggs are occasionally found that are twice as large as the normal eggs of the species. They are supposed to arise from the union of two eggs. They develop into embryos that are twice as big as normal embryos. The origin of these "giant eggs" is of peculiar interest to embryologists, and their occurrence has led to several attempts to unite two eggs by artificial means.

The inclusion of two yolks in a single shell that is not infrequently observed in hens' "eggs" is quite a different affair. These "double eggs" of the hen are only two eggs (yolks) that have been set free from the ovary at the same time, and have become enclosed in a common albumen and shell. They give rise to two embryos, but these are not united, and both die, as a rule, before hatching, although occasionally one of them—the one nearer the large end of the egg—may survive because it is so placed that this chick may make use of the air in the air chamber at the large end of the egg during the final stages of development.

Fusion of two blastulae of sea-urchins has been observed and even experimentally brought about. The results are less instructive than when the union has taken place before development begins, and are interesting only in so far as they furnish evidence as to what extent readjustments can take place after the development has already been carried forward to the blastula stage.[1]

[1] The earliest account of the union of two embryos into monstrous double forms is that of Lacaze-Duthiers, in 1875. He observed such union in the embryos of the mollusc, Philine aperta. Metschnikoff, in 1886, recorded the fusion of two or three blastulae of the hydrozoon Mitrocoma annæ. Korschelt ('95) states that the eggs in the body cavity of an annelid Ophryotrocha are sometimes fused. The union of the blastula stages of the sea-urchin observed by Morgan ('95), Driesch ('00), Bierens de Haan ('13) and Goldfarb ('13) will be considered in the text.

The union of still older parts of amphibian embryos will be considered at another time in connection with experiments relating to grafting, since in most of these cases the development has progressed so far that the problem of readjustment involves little more than the actual union of the cut surfaces that are brought together. In Triton, however, Spemann ('16, '18) has succeeded in grafting pieces of gastrula stages together, and has succeeded in incorporating a piece of one embryo into another, even when the two belong to different species. These results have been described in earlier chapters.

In general it may be said that the results obtained from fused eggs or embryos have not solved any of the larger problems of development, but they have been useful in studying special problems, and have broadened our ideas concerning some of the possibilities of regulation between two systems each alone adjusted to produce only a single individual.

Giant Eggs of Sea-Urchins

The development of giant eggs of sea-urchins has been studied by Boveri ('01, '14), Herbst ('14), and Bierens de Haan ('13). Such eggs (Fig. 184*b*) furnish an opportunity to study experimentally an interesting problem, namely, the relative influence of chromatin and protoplasm in the development of *hybrid* larvae. The giant eggs of the sea-urchin have, as a rule, a single nucleus whose surface is twice that of the surface of the nucleus of the normal egg. Twice the normal number of chromosomes are present. The origin of these eggs is unknown. It has been suggested that they may arise from a failure of the protoplasm of a young germ-cell to divide at a time when its chromosomes divide, or that they arise from the fusion of two germ-cells with subsequent fusion of their nuclei. A double cell, formed in either of these ways, would, it is assumed, grow to double the size of the normal egg. It has also been suggested that failure of one or both of the polar bodies to be extruded would produce an egg with a nucleus of double size—a nucleus with the diploid number of chromosomes, but it does not follow that such an egg would then grow to double size, since the polar bodies are formed only when the growth of the egg has come to a standstill.

Bierens de Haan ('13) records that in certain individuals and

in certain years, and at certain times in the year, giant sea-urchin eggs are not so rare as at other times. One female (Sphaerechinus) had hundreds of such eggs, while other individuals had none. These giant eggs may be fertilized, and if the sperm is much diluted, polyspermy may be avoided. The cleavage is normal, giving rise, at the 8-cell stage, to the characteristic four micromeres, etc. The embryo develops at the normal rate. Large blastulae and plutei result. The number of cells is the same as in the normal embryo, but the cells are twice as large.

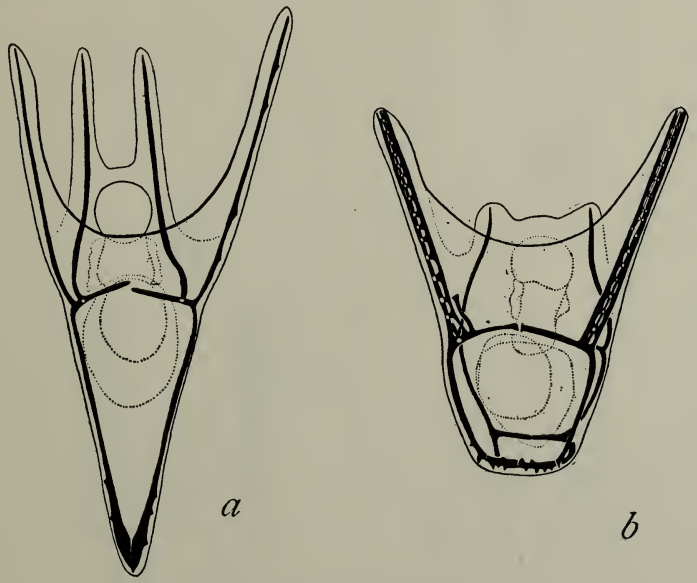


FIG. 183.—*a*, pluteus of Strongylocentrotus; *b*, pluteus of Sphaerechinus. (After Herbst.)

A giant blastula of Sphaerechinus, for example, has about 32 mesenchyme cells, and the same number is found in the normal blastulae. The normal egg of Sphaerechinus, according to Baltzer, contains 20 chromosomes, after fertilization 40. The fertilized giant egg contains, according to Bierens de Haan, 60–63 chromosomes. Forty of these have probably come from the egg (diploid) and 20 from the sperm.

Herbst ('14) has studied hybrids that have been produced by fertilizing the giant eggs of Sphaerechinus by the sperm of Strongylocentrotus. A normal pluteus of Strongylocentrotus is

shown in Fig. 183*a*, and of Sphaerechinus in Fig. 183*b*. Two hybrids from normal eggs are shown in Figs. 184a^1, a^2, and two hybrids from giant eggs in Figs. 184b^1, b^2. It is obvious at a

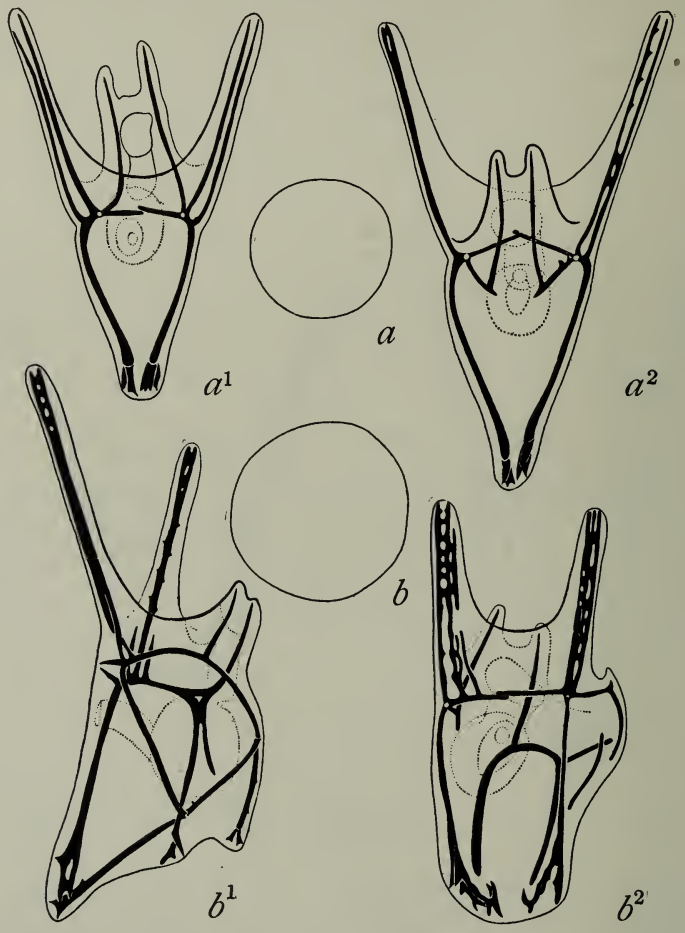


FIG. 184.—*a*, normal egg, and *b*, giant egg of Sphaerechinus; a^1 and a^2, hybrid plutei from Sphaerechinus by Strongylocentrotus; b^1 and b^2 hybrids from giant egg of Sphaerechinus by sperm of Strongylocentrotus. (After Herbst.)

glance that the latter are more like the Sphaerechinus type (Fig. 183*b*) than like the hybrid from normal eggs (Fig. 184a^2).

Herbst has analyzed in detail the characters of the larval skeleton of these two kinds of hybrids. The skeletons are quite variable, but in nearly every respect the skeleton of the hybrid

giant-pluteus is more like that of the Sphaerechinus pluteus than like that of the hybrid from normal sized eggs.

Herbst records more variability in the size of the nuclei in the giant eggs than was observed by Bierens de Haan. His measurements show that there are two, possibly three, categories of nuclei in regard to size. There are giant eggs whose nuclei have twice the volume of those of the normal nuclei, and nuclei that have four times the volume of the normal. Possibly the latter, he suggests, are tetraploid (fourfold) and represent four potential eggs fused together. On the other hand, it is possible that some of the differences are only fluctuations in size of a diploid nucleus. It will require chromosome counts to decide this question, and at present we have only those of Bierens de Haan, that, as far as they go, indicate a diploid condition. Herbst was inclined to interpret the more maternal characteristics of the hybrid giants as due to the larger amount of maternal chromatin in the nucleus. This means that the result depends on the greater influence of the larger number of the maternal chromosomes, and is in accord with genetic results in general.

Boveri ('13) obtained five giant plutei from giant eggs of Sphaerechinus fertilized by Strongylocentrotus sperm. These, he states, resembled the maternal type of pluteus more than does the hybrid from a normal sized egg. Boveri discusses the problem as to whether the maternal character of these giants is due to the greater quantity of the protoplasm of the egg, or to the double-sized nucleus. By means of the following experiment he showed that the amount of the protoplasm does not in itself affect the character of the hybrid. Some normal eggs of Sphaerechinus were broken into fragments. The nucleated fragments were then fertilized by sperm of Strongylocentrotus. Other eggs, not broken, were cross-fertilized, and then placed in Ca-free sea water. When the 2-cell stage was reached, the blastomeres were separated. Both lots were allowed to develop into plutei. An equal number (20) of the same sized plutei of the two lots were compared, i.e., those from the ½ blastomeres were compared with embryos of the same size from the fragments. Both were alike; i.e., neither showed a greater tendency to be like the paternal type of pluteus than did the other. This experiment was devised in order to test whether the amount of protoplasm of the egg, as compared with the possible importation of protoplasm by the

sperm, is the factor involved in the maternal character of the hybrid from giant eggs as compared with the hybrid from normal eggs. Now in the fragment, the sperm must bring in the normal amount of its own cytoplasm (if it does import any cytoplasm at all), while in the ½ blastomere this postulated cytoplasm has been distributed as in the normal egg. The embryo from the fragment is no more paternal than the embryo from the blastomere. The question may be asked, why was not this result equally well shown by a comparison between the hybrid from a normal egg with that from a fragment of the normal egg. The answer is that the small embryos from fragments often have a less well-developed skeleton, hence they might appear more like the paternal type which is also the simpler type in this particular case. This objection is met, however, by the experiment as planned and carried out by Boveri.

The same situation comes up again in connection with Herbst's results from cross-fertilized eggs of Sphaerechinus whose development had been already started by chemical means. These eggs (normal in size) if fertilized by sperm of Strongylocentrotus give rise to plutei that are more like the maternal type than like hybrids from normal eggs. It has been shown by Herbst ('06, '07) and by Kinderer ('14) that these treated eggs have doubled the number of their chromosomes before fertilization. There are twice as many egg-chromosomes as sperm-chromosomes with the result that the influence of the maternal chromosome is stronger than when the two kinds of chromosomes are equal in number. Since the protoplasm is the same in the two cases, it is clear that the result is due to the chromosomes, although here a possibility is not excluded entirely, namely, that the initial stimulus given to the egg by the parthenogenetic agent is responsible for the more maternal character of the pluteus; or else as Boveri has suggested the sperm cytoplasm may have become injured, or affected by the changes that have taken place in the egg before the sperm entered, hence its less efficient participation in the characters of the hybrid.

Fusion of Blastula Stages of Sea-Urchin

In cultures of the eggs of the sea-urchin, Sphaerechinus, Morgan ('95) found a few fused blastulae. The eggs had been

shaken violently two minutes after fertilization in a small tube to remove the jelly and fertilization membrane. It was noticed that the eggs of certain females fused more frequently than those of other females. Unsegmented eggs were sometimes found flattened against each other, but not fused. These, when isolated, did not unite, but were obviously placed in a favorable position for such union. The actual fusion appeared to occur in the

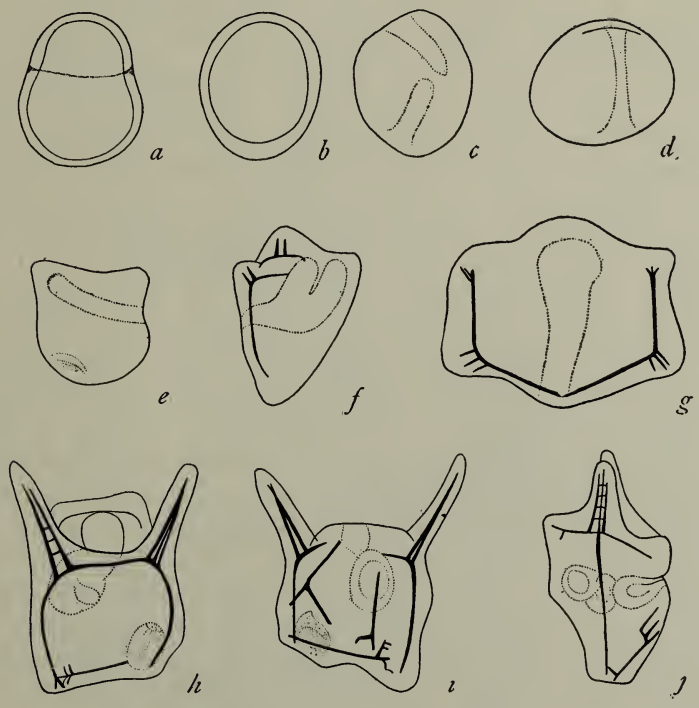


FIG. 185.—*a*, and *b*, fused blastulae of sea-urchin; *c*, *d*, *e*, gastrulae of same; *f–j*, plutei from same. (After Morgan.)

later segmentation, or else in the early blastula stages. Sometimes an oval, hollow blastula resulted (Fig. 185*b*); at other times there was a constriction between the halves (Fig. 185*a*). In both cases there was a common blastocoel. When gastrulation took place there were two invaginations as a rule (Fig. 185*c*), but when the two invaginations arose near together they united at times into a single tube (Fig. 185*d*). In this case a prismatic

larva followed (Fig. 185*e*) with one archenteron as the chief organ (Figs. 185*f*, *g*, *j*).

The plutei from such fused blastulae are nearly all abnormal, although sometimes they may superficially appear to be normal or nearly so. Closer inspection reveals in most cases traces of a second skeleton and also parts of an imperfect archenteron. (Figs. 185*h* and *i*). The results show that when fusion takes place after the eggs have divided, two imperfect embryos are produced. It is probable, therefore, that the first steps towards differentiation have already taken place, and readjustment is difficult or impossible. The double embryo approaches more nearly a single structure when the two blastulae unite in such a way that their primary axes coincide. When this happens the two invaginations may unite into a single archenteron. In most of these fused embryos, certain adjustments occur, and this is in accord with other results showing that when blastulae are cut vertically, each half may form a nearly perfect larva.

Herbst ('92) found that after eggs had been treated by lithium solutions some of the larvae and plutei united with each other. Driesch ('00) found a method by which fused blastulae of Sphaerechinus and Echinus could be produced in large numbers. The eggs were shaken to remove the membrane three or four minutes after fertilization, and were then placed in slightly alkaline sea water (NaOH, 20 per 1000). More double embryos were obtained in this way than from the control eggs in normal sea water. The time of union was not discovered, but probably took place in the blastula stage. Besides the double forms (Fig. 186*c*) Driesch found forms in which one pluteus was much more developed than the other, and also a few single giant larvae (Fig. 186*a*, *b*). In the latter, all of the material went to form one embryo.

The union of sea-urchin eggs has also been studied later by Bierens de Haan ('13). The membranes were removed from the eggs of Arbacia by shaking, and the eggs were then transferred to Ca-free sea water to which a little NaOH was added. Here they remained until the first cleavage was completed. They were then centrifuged in small tubes for 3 to 5 minutes at 30 revolutions per minute and put into sea water. Ten to forty per cent of the eggs stuck together (Fig. 187*a*, *b*). It appears from de Haan's account that the eggs do not unite until the blastula stage

has been reached, or, at least, if the cells had earlier been brought into close union, the blastocoel cavities do not unite until the blastulae begin to enlarge. Two or more embryos united. The combinations were often irregular in outline. Only the simpler unions gave rise to twins, or triplets, the rest to very abnormal

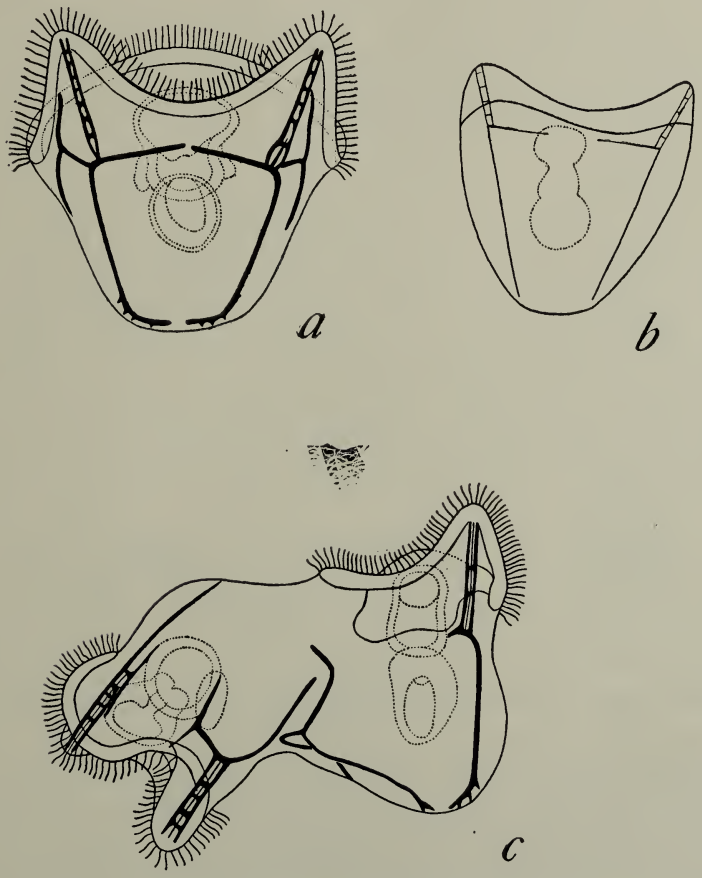


FIG. 186.—Plutei from fused blastulae of sea-urchin. (After Driesch.)

forms. Goldfarb ('13) has also described in detail double plutei of Toxopneustes ('13, '14) and Arbacia ('15). The eggs were placed soon after fertilization in sea water plus NaCl (45 cc. sea water plus 55 cc. 0.5 NaCl). Many of the eggs fused together. The eggs were left 8 to 10 hours in the solution, and were then

transferred to sea water. Fusion occurred, however, only in some of the cultures depending apparently more on the time of sojourn in the solutions than on the strength of solution employed. The solution may be isotonic, or hypotonic. Ripeness of the egg is also apparently an important factor in the result.

Bierens de Haan ('13) brought about the fusion of embryos of Paracentrotus with those of Sphaerechinus by removing the membrane or after the blastulae had escaped. In the latter case, the blastulae were first centrifuged and then brought into artificial sea water (plus KOH). He also found that the unfertilized eggs

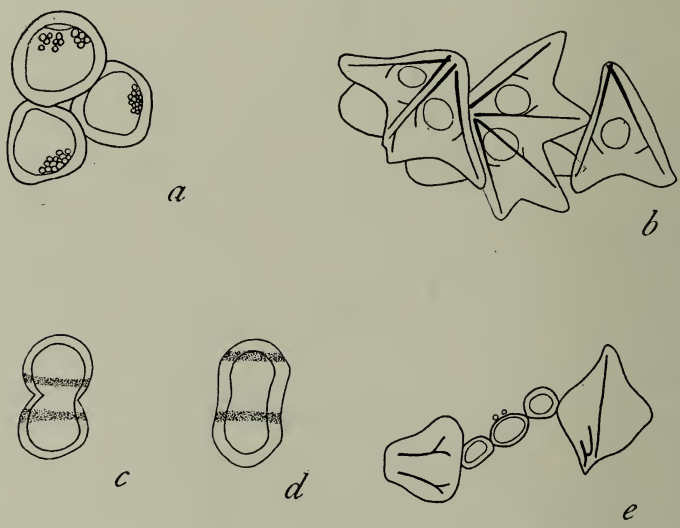


FIG. 187.—*a–e*, blastulae and plutei of sea-urchin, stuck together or partly fused. (After Bierens de Haan.)

of Parechinus if left for 40 hours in sea water often flattened against each other. The line between them sometimes disappeared and a double egg that was hour-glass in shape resulted. These became more rounded and later formed a spherical mass with two nuclei. At first the two protoplasms could be distinguished. Whether the nuclei united was not seen. On insemination no membrane was formed, but none formed on normal eggs left so long a time in sea water. The eggs were fertilized, however, and divided irregularly. Out of 40 fused eggs, only 16 formed irregular blastulae and only 8 gastrulated. These had a single gut, but went no further in their development.

More successful experiments were made with unfertilized eggs of Sphaerechinus in alkaline (NaOH) sea water. After 19 hours in the solution, several giant eggs were found; these were three-fold in 40 cases, with three nuclei; double in 35 cases, with two nuclei. From the double eggs ten double embryos were obtained of which three probably had a single nucleus from the beginning. After insemination with sperm of Paracentrotus only two irregular blastulae were obtained. Of 18 doublets from eggs fertilized by sperm of the same species, only 2 reached the blastula stage; one of these formed a single gut and a small skeleton.

Blastulae from fertilized eggs could be united by placing them in alkaline water. The blastulae (19 hours old) were made to stick together by centrifuging. They were then put into alkaline sea water with calcium. Although some of the blastulae stuck together, their blastocoels did not become continuous. Two, three or even larger groups of embryos that were stuck together often developed. They were closely fused, or even continuous at their points of contact. Successful unions were more often obtained from fertilized eggs lacking membranes. They were first put into Ca-free solution, and then removed to sea water where they stuck together in masses. Only rarely two or more of these actually fused.

Fusions of eggs of Paracentrotus (whose red band makes it possible to identify the regions of union) were also made. In cases where the two portions were united at the "vegetative pole" (Fig. 187*c*), the gut in each was turned out, or pulled out. If one embryo is united at its invagination pole to the other at some other point, the former becomes an "exogastrula" (Fig. 187*d*). Attempts to unite eggs or blastulae of two species were not successful when the ordinary methods were employed, but by mixing eggs of two species and keeping them pressed together until the blastula stage, some successful cases were obtained. Only Parechinus and Paracentrotus formed species twins. Twelve heterogenic unions were obtained. All were bizarre in form.

Bierens de Haan is sceptical regarding the formation of one giant embryo from two blastulae. He thinks that the "moment" of union is not the principal factor. It might be possible to produce a giant he thinks only in cases where two eggs unite parallel to the axis into a whole in the same way as the two blastomeres of one egg are united.

In a later paper ('14) Bierens de Haan records further results in uniting embryos of the two species mentioned above. In a few cases the two united blastulae developed as far as the pluteus stage, each largely independent of the other. In one case a part of the wall and ciliated band of the major component (that formed a nearly normal single pluteus) incorporated in itself a part of the other component. More rarely he observed that the united blastulae that had a single blastocoel, constricted apart from each other at the line of union, possibly because one species developed faster than the other.[2]

Von Ubisch ('25) by a combination of several methods has succeeded in bringing about the union of ½ blastulae of the sea-urchin, Echinus miliaris. The membrane was shaken off after fertilization, and half of the eggs were stained by the addition of a few drops of Nile-blue to the sea water. As soon as cleavage began all the eggs, stained as well as unstained, were transferred to calcium-free sea water where they formed somewhat irregular cell plates. In other cases the first two blastomeres were separated in the Ca-free sea water. When the eggs were in the 16-cell stage and the half-eggs in the 8-cell stage, numbers of stained and unstained eggs as well as the half-eggs were kept together in sea water. Most of them did not fuse but formed blastulae of whole or of half size, but a few fused, some of which combined colored and uncolored blastulae. The combinations between whole eggs offered nothing new, but several of those between ½ blastulae were of interest. For example, in Fig. 188*a*, a double gastrula is represented where the two gastrula invaginations are side by side. In Fig. 188*b* the invaginations are at right angles, one of the invaginations occurred exactly at the border between the two halves but is composed of material from one component alone. In other words despite the nearness of the gastrulating

[2] Janssens ('04) has described some peculiar giant embryos of sea-urchins that appear to come from fertilized eggs that had segmented and then fused with masses of broken down materials present in the ovaries at the time when the eggs were obtained. His description and illustrations lead one to infer that the blastulae stuck to the broken down mass of material that was spread out over the bottom of the dish. The eggs may even surround all or part of this material. When the blastula cells become ciliated, the entire mass may move about. These results resemble more the growths seen in tissue cultures than they do embryo-fusions. In some respects they recall the Chaetopterus embryos described by Lillie that come from unsegmented eggs.

region to the wall of the other half, the inturned tube did not receive any contribution from it. In Fig. 188*c* and in Fig. 188*d* embryos of full size from two ½ blastulae are shown, each with a single invagination whose wall is made up of parts of each component. In these cases the fusion may be supposed to have occurred in such a way that the two endodermal poles were in

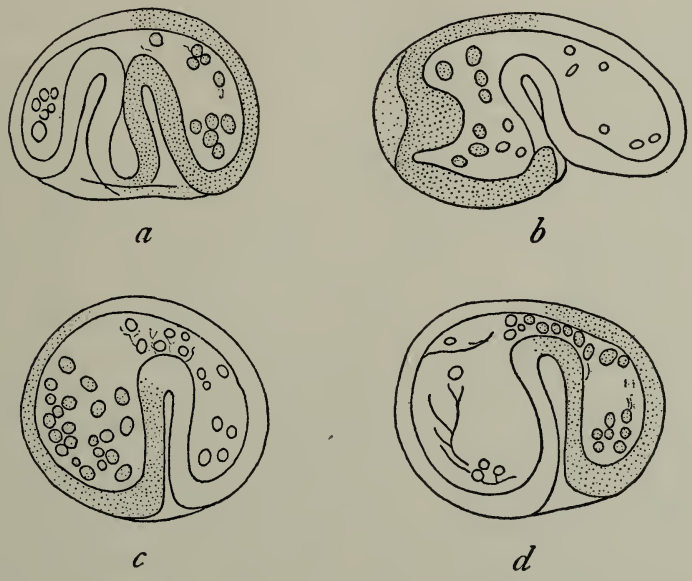


Fig. 188.—Fusion of ½ blastomere of sea-urchin (one stained in Nile-blue). Four gastrulae from fused ½ blastomeres. (After von Ubisch.)

contact and united (as in the whole embryo) to form a single tube.

These and other results of von Ubisch's are consistent with the view that normal embryos of double size from the fused blastulae, or a normal embryo of full size from the two fused ½ blastulae, are formed only when the two blastulae are united so that similar regions have the same orientation. This applies, so far as demonstrated, strictly only to the gastrula axis. To what extent the bilateral structure of such a double combination, where fusion took place after cleavage was finished, can be adjusted after fusion is not evident from these cases.

Double Eggs of Ascaris

The eggs of the thread worm of the horse, Ascaris megalocephala, have furnished interesting cases of fusion. The eggs are said to unite in some cases before, in other cases after fertilization. Occasionally, these double eggs appear to give rise to single giant worms. Sala ('93, '95), zur Strassen ('96, '98) and Kautzsch ('13) have described the process of fusion and its subsequent results, but their accounts differ in certain important points. For instance, there is some doubt as to the time at which the union takes place. Sala suggests that some of the unions are due to incomplete separation of the oögonia in their last divisions. Such eggs, with two nuclei, would, he thinks, behave like normal eggs. They would be expected to form four polar bodies, two from each nucleus, and be fertilized by one sperm. They would then contain a triple set of chromosomes. The development of such eggs was, however, not followed. In other cases double or triple eggs may be produced, according to Sala, by the action of cold on the eggs. The jelly formation is delayed, or, if formed, it remains soft and eggs may stick together and even become united by bridges of protoplasm. Later, the fusion may go farther. Such unions, he supposed, take place either before or just after fertilization. The number of sperms that enter the eggs is variable. The eggs later die without forming embryos.

Zur Strassen believes that the union takes place between separate eggs. He found that a low temperature might increase the number of unions, but was not the only cause of such unions. The union takes place usually between naked eggs, i.e., before the jelly is formed. One sperm may enter. The polar bodies from each nucleus are extruded, sometimes at opposite sides, and sometimes near together. Zur Strassen believes that eggs may also unite even after their membranes have developed. The membranes stick together, fuse, and a canal develops between the two. Through this canal the protoplasm from one egg passes and unites with the protoplasm of the other egg (Fig. 190*a*, *b*). The two eggs are then supposed to flow together and unite into a single more or less spherical mass.

Kautzsch states that a single interpretation will cover all the cases observed by Sala and by zur Strassen. He points out that if the fused eggs are arranged in the order of their stages of

polar body formation, the youngest stages are always those without a membrane. In Fig. 189*a* the first two polar bodies are being given off from a double egg. One spermatozoön is present. A slightly later stage of another egg is shown in Fig. 189*b;* both polar bodies are formed near together, and two sperms are present. In still another egg, Fig. 189*c*, the polar bodies have been given

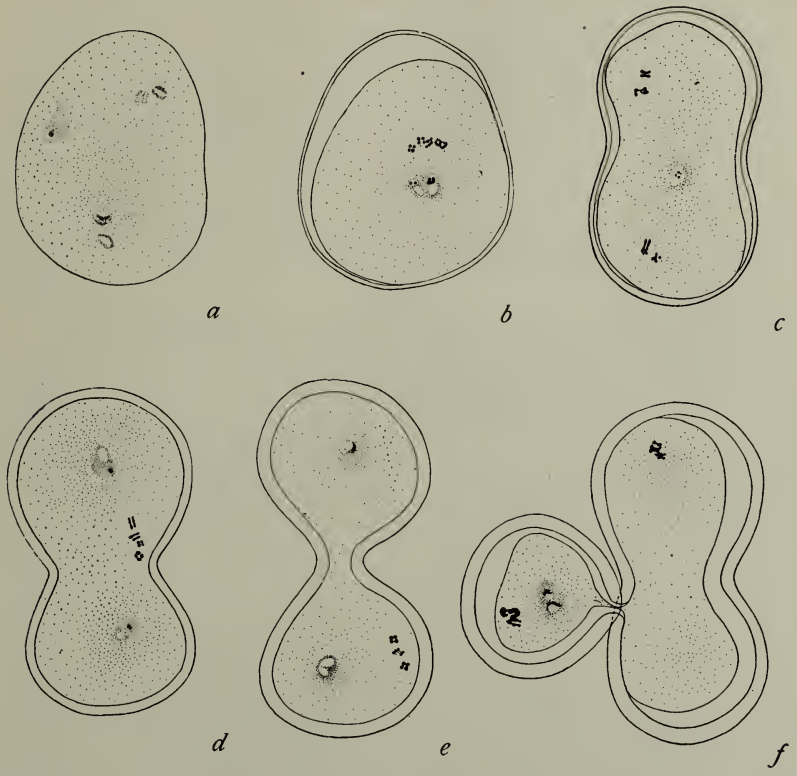


FIG. 189.—Double eggs of Ascaris, showing polar bodies (diad-groups of chromosomes) egg-nuclei (also as diads), and sperm-nuclei. (After Kautzsch.)

off by each nucleus, but at different poles. Only one sperm is present. In another egg, Fig. 189*d*, all four polar bodies are near together in the constricted region; two sperms have entered. This egg is dumb-bell-shaped. In contrast to the last two cases the polar bodies of the egg represented in Fig. 189*e*, lie at the surface in one of the dumb-bell-shaped combinations, and the two sperm-nuclei lie one in each of the rounded ends of the double

egg. The union of three eggs is shown in Fig. 189*f*. The polar bodies of one egg of the three lie in the dumb-bell-shaped (right-hand) part, and the polar bodies of the other two eggs lie in the left-hand portion that is now constricted off from the dumb-bell part. Two sperm are present in the smaller part. Kautzsch

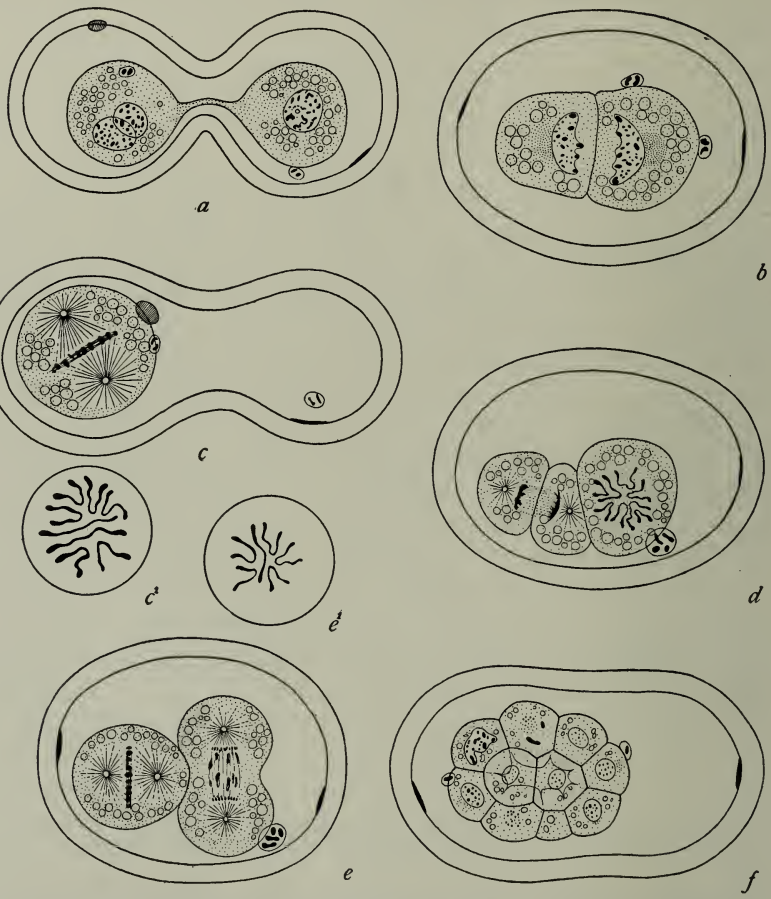


Fig. 190.—Fused eggs and cleavage stages of Ascaris. (After Zur Strassen.)

argues that in this case it is improbable that one nucleus could have passed through the narrow connection to form its polar body with the left-hand egg, and that it is more probable that, after two of the fused eggs had given off together their polar bodies, a constriction appeared that formed the bridge between them. In

other words, he thinks that in all these cases the eggs were at first more or less closely fused, and that after extrusion of the polar bodies and fertilization, the halves tend to round up again. This gives rise to the dumb-bell combinations seen in so many cases. Thus he reverses the order of events postulated by Sala and zur Strassen for many of the double eggs. In favor of Kautzsch's view are those cases where the polar bodies are given off near each other, for it does not seem probable that they could have been secondarily brought into this position by a union of the eggs after they had been extruded. Also in favor of his interpretation is the improbability that union could take place after the jelly and fertilization membranes had been formed. Both zur Strassen and Kautzsch agree that in later stages, after segmentation has taken place, the two eggs may sometimes come together to form a more nearly oval or spherical embryo (Fig. 190*b*, *c*).

The segmentation stages of some of the double eggs which fused at an early stage have been followed by zur Strassen. The segmentation of many of the double eggs shows generally great irregularity arising from the presence in them of two separate egg-nuclei and one or two sperm-nuclei, but sometimes the pattern is quite normal. When two sperm-nuclei are present, two spindles or a multipolar complex of spindles develops that leads to irregularities in the distribution of the chromosomes, as well as to irregular division of the cytoplasm. When one sperm-nucleus is present, a regular spindle may develop, whose metaphase plate (Fig. 190) contains the six chromosomes derived from the three nuclei present. What percentage of such eggs develops as a single unit is not known, but since later (Fig. 190*e*) giant eggs with normal cleavage pattern are sometimes found it is probable that on rare occasions the development proceeds in quite a normal way (Fig. 190*d*, *e*, *f*). It is also possible that in other cases when two sperm have entered, a normal cleavage may take place provided a single spindle develops. The presence of 8 chromosomes in such an egg (Fig. 190c^1) is evidence that two sperm have entered. It follows that both triploid and tetraploid embryos may develop into giants. As stated above, zur Strassen records finding a giant embryo (Fig. 191*b*) within one egg-membrane, and the size of the double embryo as compared with the normal, as well as the size and shape of the membranes, leaves no doubt as to its double

origin. The results show that some of the unions at least are of such a kind that the protoplasm of two eggs, and probably

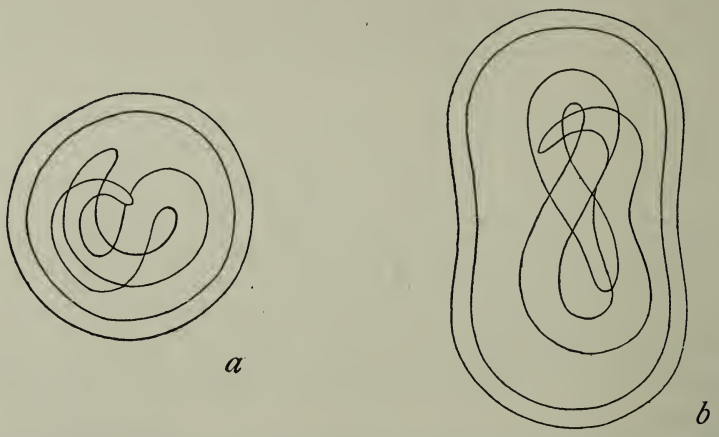


Fig. 191.—*a*, normal, and *b*, giant egg of Ascaris within membrane. (After Zur Strassen.)

also the nuclei of the two eggs, have united and produced a single embryo of double size.

Double Eggs in Nemerteans

Giant embryos of the nemertean, Lineus ruber, arising from the fusion of two eggs, have been described by Nusbaum and Oxner ('13). The eggs are laid in cocoons, two or more eggs in each. They may fuse before cleavage (Fig. 192*a*) during cleavage, or in the blastula stage (Fig. 192*b*). The eggs that fuse before cleavage may contain two or more nuclei, but sometimes only one. When more than two eggs fuse, the cleavage is so irregular that embryos do not develop, but when only two eggs fuse, gastrulation (Fig. 192*d*, *e*) may take place and a giant embryo may be formed. Two fused eggs may have a common blastocoel and a single archenteric invagination. These appear to give rise, at times, to single giant embryos. When partial fusion takes place between the two blastulae each of them may invaginate separately. The double-headed embryos that have been found (Fig. 193) may be produced in this way.

The development of whole embryos from parts of eggs, or

from isolated blastomeres, or even from pieces of blastulae of another nemertean (Cerebratulus) indicates that the blastomeres of these worms are little differentiated, or else have extensive powers of "regulation." Hence, the results of fusion of two

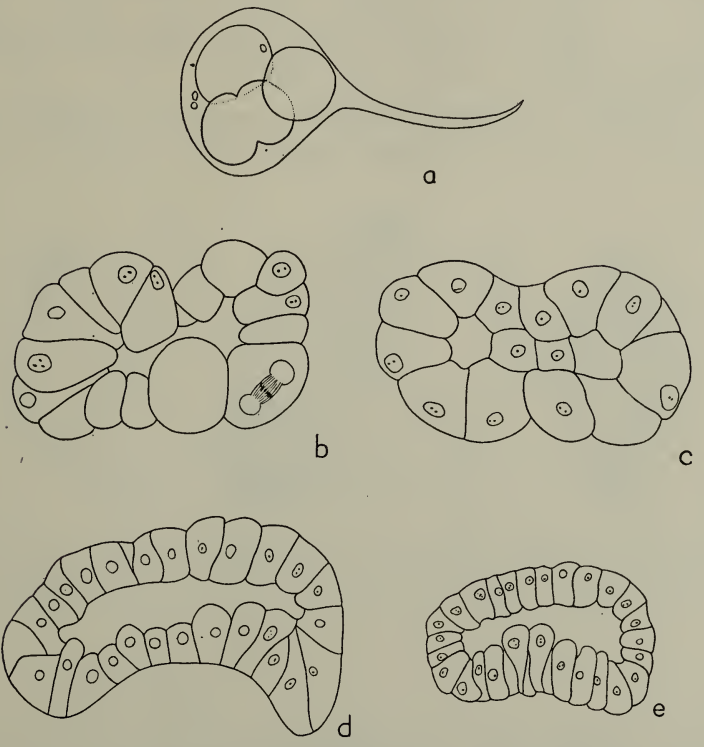


FIG. 192.—*a*, capsule containing two normal and one fused egg of Lineus; *b* and *c* fused segmentation stages; *d* and *e*, older stages of fused eggs. (After Nusbaum and Oxner.)

eggs or embryos are entirely consistent with the known possibilities of these eggs.

GIANT EMBRYOS OF TRITON

The union of two eggs of Triton to form a single giant embryo has recently been brought about by Mangold ('20). The eggs were removed from the jelly as they were passing into the 2-cell stage. In the absence of the membrane the egg flattens and the first two blastomeres at the height of the division period separate

widely until they are nearly tangent to each other. One such egg is then lifted up and laid across another one in the same stage (Fig. 194*a*). As soon as the four blastomeres begin to draw together they flatten against each other. This union becomes more and more intimate as the cleavages proceed (Fig. 194*b*). Gastrulation takes place later (Fig. 194*c*), and a single embryo may be formed (Fig. 194*d*), or else two or even three embryos united together may result.

In order to understand the different possibilities involved when two eggs of Triton are brought together it is necessary to take

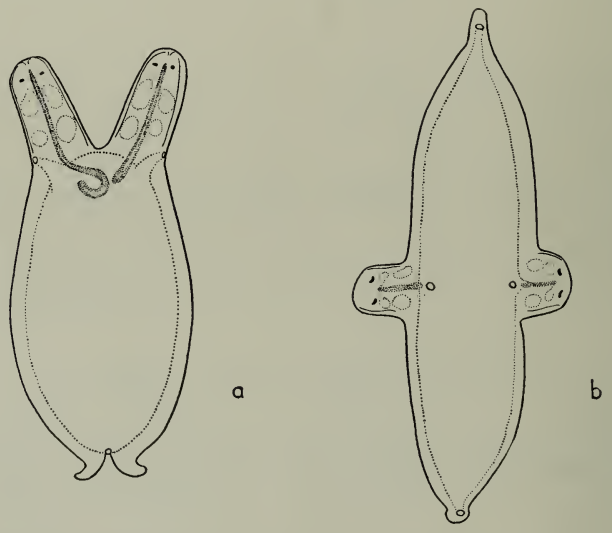


Fig. 193.—Two double-headed embryos of Lineus from fused eggs. (After Nusbaum and Oxner.)

into account the fact that the first cleavage plane is sometimes median and at other times frontal (i.e., across the median plane). By means of the following diagrams (Figs. 195-6-7) the possible relations of the fused eggs to each other are shown (one egg is stippled in each case). The first plane of cleavage is indicated by the straight continuous line, and the future position of the dorsal lip of the blastopore (by which the median plane is indicated) is represented by the black crescent. The two small circles to the left represent the kinds of embryos involved with respect to the first cleavage plane. The two larger circles to the

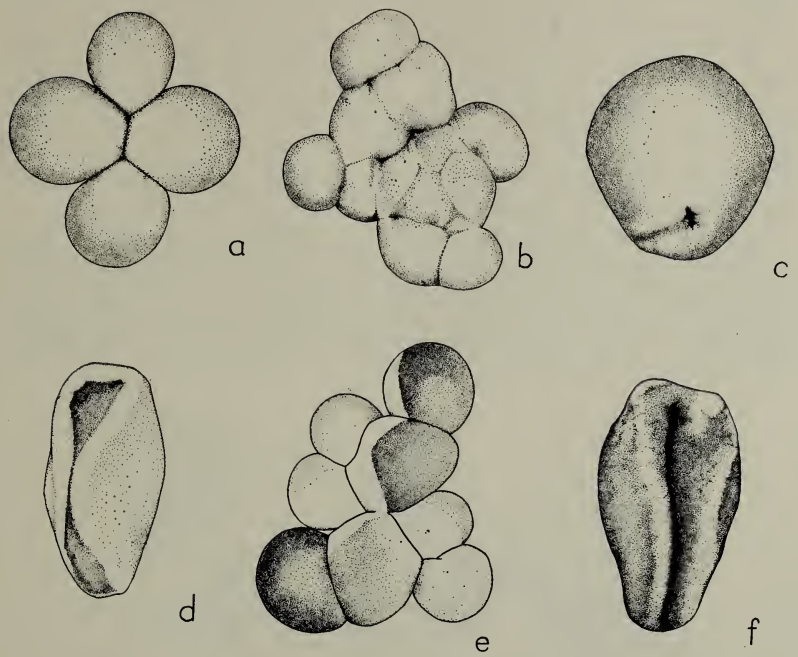


FIG. 194.—*a*, two eggs of Triton, in two-cell stage, laid across each other; *b*, later cleavage stage of *a*; *c*, single gastrula and, *d*, neurula stage of same; *e*, another cleavage stage of two eggs united as in *a*; *f*, neurula stage of same. (After Mangold.)

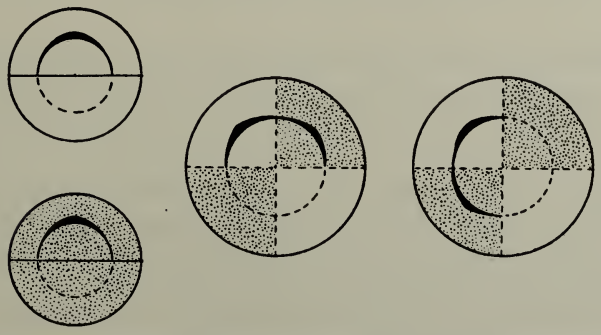


FIG. 195.—Diagrams showing method of union of two eggs of Triton in two-cell stage, as in Fig. 194; *a*, the first cleavage of each is at right angles to median plane (frontal). The two smaller figures to the left show the two corresponding gastrula-stages (first cleavage plane as straight line). The two larger figures to the right show the two possible relations of the dorsal lips after union. (After Mangold.)

right represent in each case the result of the combination of the former to produce a giant gastrula.

In Fig. 195, the first furrow in each embryo is frontal. When eggs of this sort, in the 2-cell stage, are laid across each other the two possible relations of the future blastopore are represented by the two larger circles to the right. In each case the rim of the blastopore forms a continuous half-circle of double size. Here the axes of the two components coincide as far as possible.

In Fig. 196 the first furrow in each embryo is median. When two such eggs in the 2-cell stage are laid across each other, the two possible relations are shown by the larger circles to the right. Here there is one normal-sized dorsal lip made up of halves of

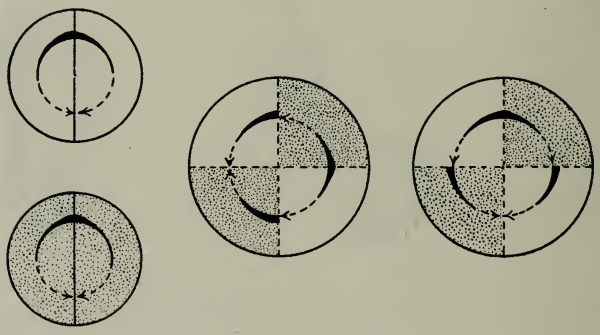


FIG. 196.—Like Fig. 195, but here the first cleavage of each egg had coincided with the median plane. (After Mangold.)

each embryo, and two half dorsal lips in the other hemisphere. The axes of the components are not in the same direction. There is only one whole dorsal lip formed by the juxtaposition of two half-lips.

In Fig. 197 the first furrow in one embryo is frontal and in the other embryo median. When two such eggs in the 2-cell stage are laid across each other, the two possible relations are indicated by the larger circles to the right. In the first of these a single dorsal lip is continuous at its edges with the two half-lips of the other embryo. The axes of the two are nearly in the same direction. In the second, the single dorsal lip is isolated from the two half-lips of the other embryo, and the axes of the components are approximately reversed.

There is a very high mortality, but this is also true for single

eggs that have been removed from the jelly membrane. Failure of the giants to develop is due, no doubt, in part to their exposure, but probably also in some cases to difficulties resulting from the enforced union of two eggs and the resulting maladjustment of their parts. In two cases, nevertheless, single, normal embryos of giant size were obtained. One of these came from two eggs of Triton taeniatus (Fig. 194*d*), and the other (Fig. 194*e*, *f*) from an egg of this species united to the egg of another species, Triton alpestris. It was not possible to determine the nature of the special kind of combination that gave these results, but it seems not improbable that they came from such a union as that shown in Fig. 195, or from the first union shown in Fig. 197.

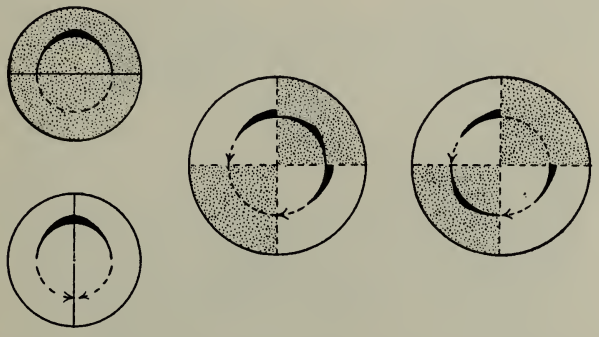


FIG. 197.—Like Fig. 195, but here the first cleavage plane of one egg was frontal and the other median. (After Mangold.)

Mangold also describes another monstrous embryo in which three anterior ends or heads were united into a single giant. Such an embryo is expected to arise from some of the other unions shown in the diagrams.

These results, although somewhat meager, show, nevertheless, the possibility of forming a single embryo of Triton by the union of two eggs at the 2-cell stage. They are, moreover, in accord with the results obtained from rotated blastomeres of these same eggs.[3] The outcome suggests that even although at the 2-cell

[3] Mangold also produced single embryos of normal size by uniting halves of two eggs. The first two blastomeres were first separated from several eggs, then later when each of these was dividing, two of them were laid across each other. They united into a single embryo. The possibilities of combination are much the same as those described above for whole eggs, depending on the position of the first plane of division with respect to the axis of the embryo.

stage the future axes of the embryo are determined, yet an adjustment is possible if the general orientation is the same in each component.

Double Eggs that Are Not Giants

To what extent in other animals two eggs may fuse to produce giant embryos is not known, but there is evidence to show that embryos may arise that are not giants but which, nevertheless, owe their origin to the fusion of two eggs; and it is also quite certain that many monstrous forms that have double structures do not arise from fused eggs. The latter situation may be first considered. There is no evidence, for instance, that two headed

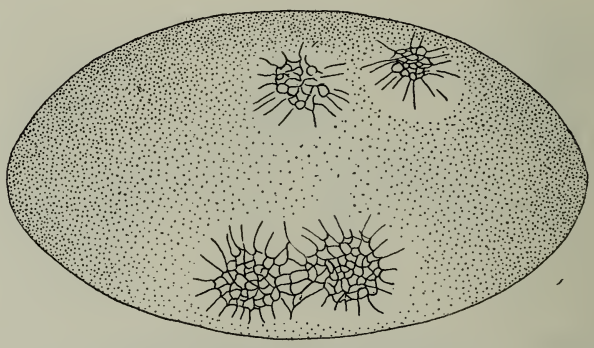


Fig. 198.—Egg of Tropidonotus, with several blastoderms. (After Wetzel.)

fishes, or chicks, or turtles come from fused eggs. It is true there is no evidence to show that they do not arise in this way, unless the normal size of the eggs furnishes such evidence. There is in fact one case where four blastodermic areas (Fig. 198) have been found (Wetzel '00) on the egg of a snake (Tropidonotus natrix), and this at least suggests an earlier four-nucleated condition, but such observations are rare compared with the frequency of double embryos in other vertebrates.

Several writers have suggested that double chicks may arise from the entrance of more than one sperm into the egg, but the entrance of several sperms appears to be a normal occurrence in the hen's egg (Patterson '10). The extra sperms take no part in the later development. The not infrequent occurrence in the hen's egg of two yolks in one shell is due, as already mentioned,

to the liberation of two eggs from the ovary at the same time, which, passing one behind the other down the oviduct, become enclosed in a common albumen and shell. They do not fuse and do not give rise to double monsters.[4] The multiple embryos of the armadillo have been shown to arise from a single egg (Patterson and Newman) by a sort of duplication or "budding" in a stage following cleavage. There are no grounds for assuming

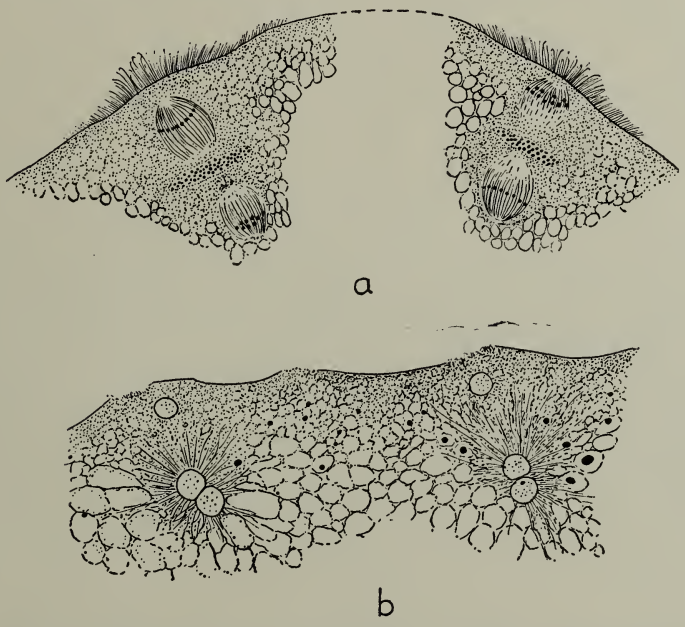


FIG. 199.—Two sections of egg of moth, Abraxas: *a*, two polar bodies are present, one from each egg-nucleus; *b*, union of two sperm-nuclei with the two egg-nuclei, one with each. (After Doncaster.)

that the eggs have more than one nucleus, in fact, only one is figured in all the normal eggs that have been described.

The multiple embryos of certain parasitic wasps have also been traced (Bugnion '91, Marchal '04, Silvestri '06, Patterson '13, '15, '17, '18, '21) to single eggs, each with a single nucleus. The mass of cells resulting from cleavage breaks up later into a chain of embryos.

[4] G. H. Parker ('06) has reviewed the literature on "Double hens' eggs." Raymond Pearl ('10) has described "A triple-yolked egg" and given references to other records of similar cases.

There is one case in insects where eggs with two nuclei have been observed and where the changes that take place in them have been followed. Doncaster ('14) found in one strain of the moth, Abraxas, that certain individuals contained eggs with two separate nuclei. Each nucleus forms its polar bodies (Fig. 199*a*) and each reduced nucleus then apparently unites with a separate sperm-nucleus (Fig. 199*b*). The observed entrance of more than one sperm into insect eggs, that has often been recorded, makes this double fertilization not so unusual as might appear at first thought. These doubly nucleated eggs of Abraxas produce a single embryo normal in size, since the eggs themselves are not larger than normal ones. How these eggs arise is not known, but the conditions that prevail in the early germ track in insects, where a group of cells, derived from a single oögonial cell, becomes enclosed in a common follicle, would seem favorable to such a union. Furthermore, only one cell in each group usually becomes the egg while the rest remain as nurse cells. Although we do not know what conditions bring about the specialization between these apparently identical cells, it is customary to assume that the position in the group of one of the cells determines that it becomes the egg, hence it is easy to imagine that two cells failing to divide completely might reunite into one cell with two nuclei and become a double egg. Failure of such eggs to become giants may be explained by the restriction of the tube in which the egg is confined and through which it passes during its growth stages. Possibly also in such cases the failure of double eggs to grow to double size may be due to presence of only the normal number of nurse cells which supply a large part of the materials for growth.

Since it has been shown in moths that the female is heterozygous for a sex chromosome, it is evident that if two eggs should unite and each nucleus remain separate from the other, one nucleus after extrusion of the polar bodies might be left with the Z chromosome (the W chromosome being extruded) while the other nucleus might be left with its W chromosome (the Z being extruded). Since all the sperms are alike, i.e., each carries a Z, it is obvious that a gynandromorph would arise, namely, an individual that is male on one side and female on the other. It is probable that some of the bilateral gynandromorphs that have been found in moths and butterflies may arise in this way. They are not so much double embryos, as two half-embryos, one male,

one female united into one. There are still other ways in which bilateral gynandromorphs may arise, and their occurrence does not necessarily mean that all gynandromorphs arise from binucleated eggs; but when a female is heterozygous for genes other than those carried by the sex chromosomes, it may be possible to show by analysis that double nucleated eggs must have been the source of the mosaic individual that appears. There are, in fact, two cases of this kind described by Toyama in the silkworm moth both from the same brood. Here, as shown by the analysis of the situation (Morgan '07, '14, '19), the two gynandromorphs must have come from a double nucleated egg. There are also a few other cases in moths where this explanation is probable.

In the vinegar fly, Drosophila melanogaster, in which a large number of mutant races are known, and in which gynandromorphs are of frequent occurrence, there are occasional cases where the usual explanation of "elimination," in an embryonic division of an X-chromosome from a dividing nucleus, does not apply, but where the results are in full accord with the assumption of a binucleated egg (Morgan and Bridges '19, Morgan, Sturtevant and Bridges '23). At present, there is lacking the cytological evidence that is necessary before such cases can, with certainty, be referred to an egg with two nuclei, but the genetic evidence leaves little doubt as to their interpretation.

CHAPTER XXI

THE INFLUENCE OF PRESSURE ON CLEAVAGE, AND THE EFFECT OF CHANGES IN THE CLEAVAGE PLANES ON DEVELOPMENT

Pflüger discovered in 1884 that when the frog's egg is slightly compressed before cleavage between two glass plates, the direction of the first three cleavage planes is at right angles to the plane of the compressing plates. He also found that normal embryos develop if the compressed eggs are released. He used this result as an argument in favor of the view that the material of the egg is isotropic, and that it is a matter of indifference how this material is cut up by the cleavage planes to form the building blocks of the embryo. The same method, when applied to other eggs, has given results similar to Pflüger's in so far as the influence of the pressure on the direction of the cleavage planes is concerned, but the resulting effects on the formation of the embryo have been found to be different in eggs of different types. The outcome runs strictly parallel to the results from isolated blastomeres.

A description of the changes induced by compression may be given first, and be followed by a discussion of the hypotheses advanced to account for the way in which the cleavage is affected. The bearing of the facts on certain theories of development will then be considered.

The Effect of Pressure on the Sea-Urchin Egg

The eggs of the sea-urchin lend themselves readily to pressure experiments because, owing to their viscosity, they may be flattened without bursting.

Driesch ('92) carried out pressure experiments with the eggs of Echinus, and later Morgan ('93), Ziegler ('94) and others have made similar experiments. Fertilized eggs are placed on a

glass slide in a small drop of water. On each side of the drop, a hair, or a piece of thin glass is laid to act as a support to the cover-slip when it is placed over the drop. The cover-slip spreads the drop and compresses the eggs. If too much water is at first present, the cover-slip may float on its surface and the eggs may fail to be compressed. But the excess of water may be withdrawn until the eggs are compressed. The degree of flattening is then determined by the thickness of the supports.

More elaborate methods have also been used. A compressorium consisting of two glass plates, a thicker, supporting-one below, and a thin cover-slip above carried in a ring of metal offers certain advantages. The distance of the plates apart can be regulated by set screws. In the best apparatus a stream of water can be kept flowing between the plates. The eggs remain alive for a longer time when oxygen is brought to them, and the carbon dioxide removed by the stream of water.

The first cleavage always appears in the compressed eggs at right angles to the compressing plates, i.e., in the direction of the pressure (Fig. 202*a*). The second cleavages lie at right angles to the first (Fig 202*b*). The third cleavages (giving 8 cells) also come in at right angles to the plates (Fig. 201*a*), and more or less parallel to the first cleavage plane, although there is much variation in the location of these planes. The fourth cleavages (giving 16 cells) are also at right angles to the plate in most of the cells, and in general, may be said to be at right angles to the last cleavage planes (Fig. 201*b*, *c*). If the egg is not much flattened, some of these later cleavages may be not strictly at right angles to the plates, but come in more or less parallel to them, especially at the edges of the plates. If the eggs are kept alive for a long time by supplying them with fresh water (oxygen), flat plates of thirty-two and even sixty-four cells may be produced (Fig. 201*d*, *e*).

If at the two-, four-, or eight-cell stage the pressure is released, the eggs become spherical after a time, and the next divisions may be in a plane that would have been parallel to the compressing plates, thus producing two tiers of cells (Fig. 200*g*). Further divisions, not fully described, lead to the development of hollow spherical blastulae. These may gastrulate and produce normal plutei.

Inasmuch as it is difficult to detect the pole of the egg of the

sea-urchin if its jelly has been removed, no attention was paid in most of the work to the relation between the planes of cleavage and the axes of the egg, but even if the first division passes through the pole, as it does in the normal egg, some of the subsequent cleavages must be in planes different in sequence from the corresponding cleavage planes of the normal egg. Since normal embryos develop from many or all (?) of these compressed eggs, it follows that the sequence of the cleavage is not essential

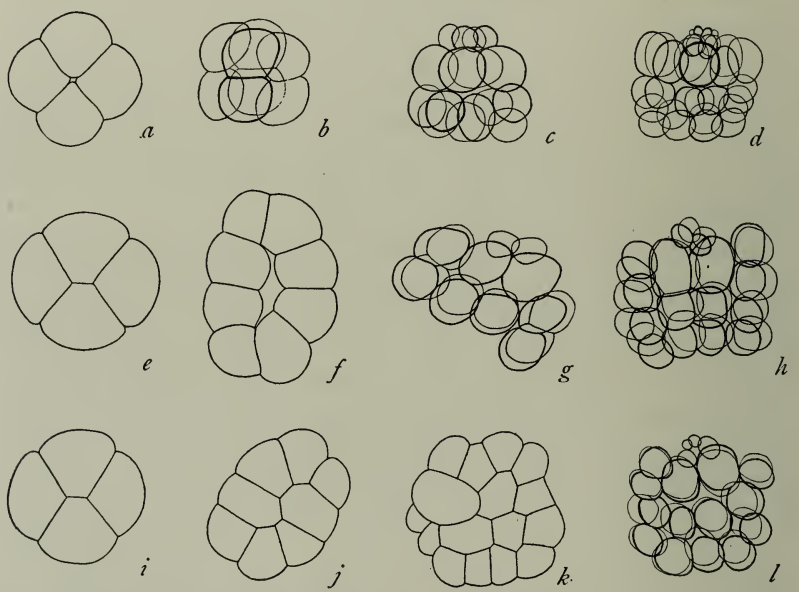


FIG. 200.—*a–d*, normal cleavage stages of sea-urchin egg; *e* and *f*, cleavage under pressure; *g* and *h*, cleavage of same after removal from pressure; *i*, *j*, *k*, cleavage under pressure; *l*, cleavage after release from pressure. (After Driesch.)

to the formation of a normal embryo. In other words, it appears to be a matter of secondary importance how the egg of the sea-urchin is cut up into smaller cells.

Despite the difficulty of identifying the pole of the compressed sea-urchin egg there is a method by which the relation to the pole of the cleavage planes of this egg under pressure may be detected. In Arbacia (as well as in other sea-urchin eggs) the segmentation nucleus comes to lie, after fertilization, in the primary axis, and somewhat nearer to the funnel in the jelly that represents the pole of the egg. After the first cleavage, the two

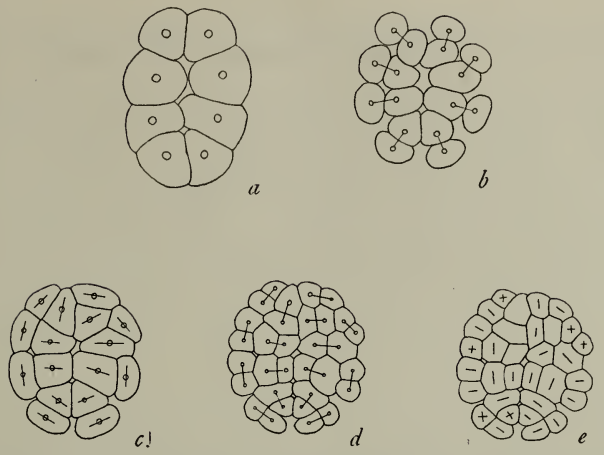


FIG. 201.—Egg of sea-urchin under continuous pressure kept alive by a stream of sea water. (After Ziegler.)

nuclei of the first two blastomeres also lie nearer to the same pole (Fig. 202*e*). In the 4-cell stage (Fig. 202*f*) they are still excentric. After the third, equational, cleavage (Fig. 202*g*) it is no longer possible to tell whether the nuclei are still excentric. At the fourth cleavage (Fig. 202*h*) when the micromeres are formed it can be seen (if the egg has been kept in one position throughout its cleavage), that the micromeres lie at the pole that is opposite the funnel in the jelly, the so-called micropyle.

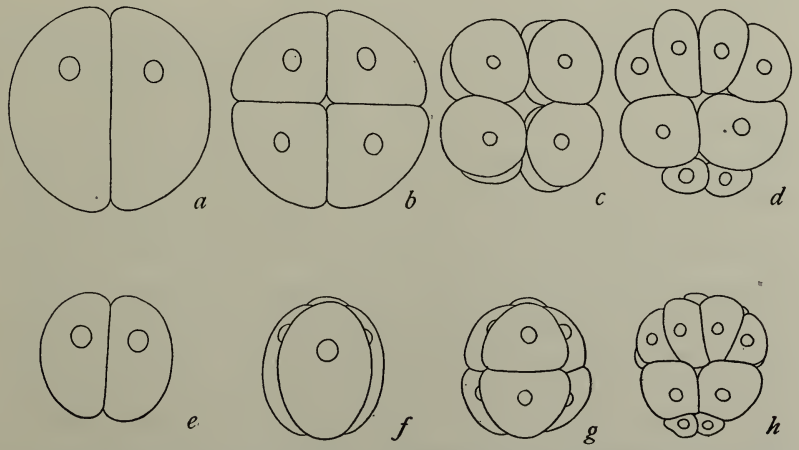


FIG. 202.—*a* and *b*, cleavage of egg of sea-urchin under pressure; *c* and *d*, cleavage after release from pressure; *e–h*, normal egg drawn to same scale. (After Morgan.)

Eggs of Arbacia, either in the 2-cell stage, or just before the first division, were compressed (Morgan, '93), and those eggs in which the nucleus lay nearer to one pole were selected for observation (Fig. 202*a*). The next cleavage came in at right angles to the first, giving four equal or nearly equal cells. The compression was then removed gently by adding more water without disturbing the position of the eggs. The third cleavage came in at right angles to the two preceding ones, and parallel, therefore, to the plates (Fig. 202*c*). At the next division the four micromeres formed at their normal pole, and the four blastomeres of the opposite hemisphere divided equally as in the normal eggs (Fig. 202*d*). It may be said, therefore, that the second cleavage under pressure is in the position of the third cleavage plane, while after removal of pressure the normal second cleavage plane has reappeared before the micromere cleavage takes place (the fourth cleavage). On the other hand nothing is gained by attempting to identify the cleavages in this way.

The micromeres of these eggs develop at the crossing point of the first and third cleavages instead of at the crossing point of the first and second cleavages as in the normal egg. The essential fact is that, when the micromeres appear, they are formed from that part of the egg from which they develop in the normally segmenting egg. In other words: the distribution of materials at the 2-cell stage (or perhaps even before that time) is such that the micromere pole is predetermined.

This conclusion is further substantiated by another observation. In one egg, that had reached the 4-cell stage under compression as above, and was then released from pressure, two micromeres were formed at once at their proper pole, while the blastomeres of the opposite hemisphere divided equally by radial divisions. Thus, a typical ½ 16-cell stage was produced with two micromeres and two macromeres and four mesomeres. The characteristic division, that takes place normally, when the eight blastomeres divide into sixteen, took place one division earlier. It is obvious that those conditions that at the normal 8-cell stage lead to the formation of the typical micromere division are already present at an earlier one. It is not, then, so much the sequence of the divisions that is primarily significant as the presence of differences in different regions of the egg.

The Effect of Pressure on the Egg of the Frog

As already stated, the first experiments on compression were made by Pflüger on the frog's egg. The striking difference in color between the two hemispheres makes it possible to determine the orientation of the egg with respect to the compressing plates. Pflüger showed that, when the frog's egg is compressed in its polar axis, the first cleavage is always at right angles to the plates and through the pole (Fig. 203*A*). The second cleavage is also at right angles to the plates and to the first cleavage, and

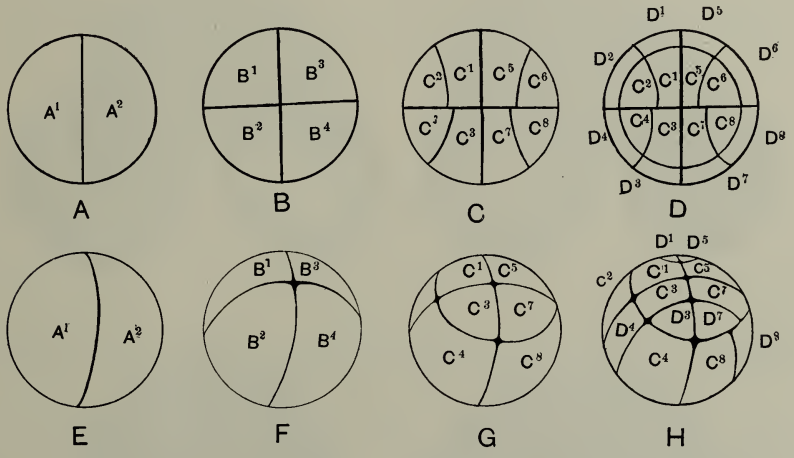


Fig. 203.—Diagrams of compressed egg, *A–D*, and of normal egg, *E–H*, to show the relative distribution of the nuclei in the two types. (After Morgan.)

through the pole (Fig. 203*B*). Both cleavage planes correspond in position to those of the normal 4-cell stage. But the third cleavages, which are also at right angles to the plates, and generally parallel to the first plane of cleavage (Fig. 203*C*), are not in the same position as is the third cleavage of the normal egg (203*G*). Eight cells result. If the pressure is then removed, the eggs round up and continue to divide (Fig. 203*D*). They produce normal embryos. If kept still longer under pressure, the next cleavages (or some of them) may still be vertical to the compressing plates, as Born has shown.

Hertwig ('93) and Born ('93) repeated Pflüger's experiment in which the frog's egg was compressed from the pole. The first

three planes were at right angles to the compressing plate (Fig. 204*a*, *b*). Eggs were also compressed from the side. The plates were then held in a vertical position so that the pole of the egg was turned upwards. The first division is vertical, at right angles to the plates, and through the pole (Fig. 205*a*). The second division is horizontal and at right angles to the first. The two black cells are, as a rule, smaller than the other two. The third division of the smaller cells is vertical and parallel to the first plane. The third division of the larger cells is often into unequal parts, but at right angles to the plates.

In some cases the first cleavage starting at the poles, passed to one side, and the second division, at right angles to the plates, divided the first two cells into unequal parts (Fig. 205*c*, *d*).

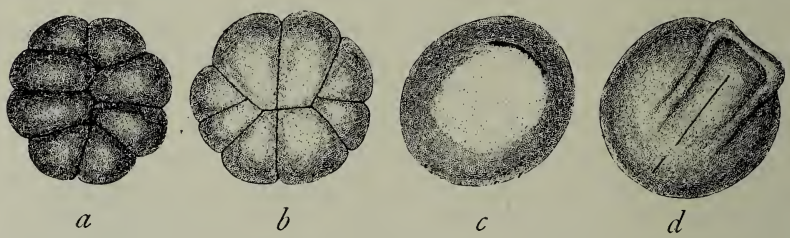


FIG. 204.—Cleavage of frog's egg under pressure: *a*, eight-cell stage, as seen from black pole; *b*, as seen from the yolk pole; *c*, beginning of gastrulation; *d*, neurula stage. (After O. Hertwig.)

Normal embryos develop from these plates of cells if the pressure is removed.

In none of these cases was the relation of the first and second cleavages to the gray crescent recorded. It is probable, nevertheless, that some of the laterally compressed eggs lay with the crescent against one of the plates, and that others lay with the crescent at or near the edge. If all these compressed eggs produce normal embryos, as seems to be the case, it follows that it makes little difference, so far as normal embryo-formation is concerned, how the cleavage takes place in regard to the crescent.[1]

The position of the blastopore is recorded in some of Hertwig's experiments. It appears at or near the edge, but it is possible

[1] This statement is in agreement with other observations (Roux, Morgan, Brachet), namely, that the first cleavage of normal eggs does not always pass through the middle of the gray crescent. Normal embryos also develop from these eggs.

that the egg may shift between the plates as the dorsal lip develops so that it is brought into this position. This is, however, not established.

There are a few experiments of Hertwig's in which the compressing plates were oblique, and the egg became wedge-shaped. The cleavage of such eggs follows, in a general way, the same plan as when the plates are parallel. The results offer little additional evidence of value to that already recorded.

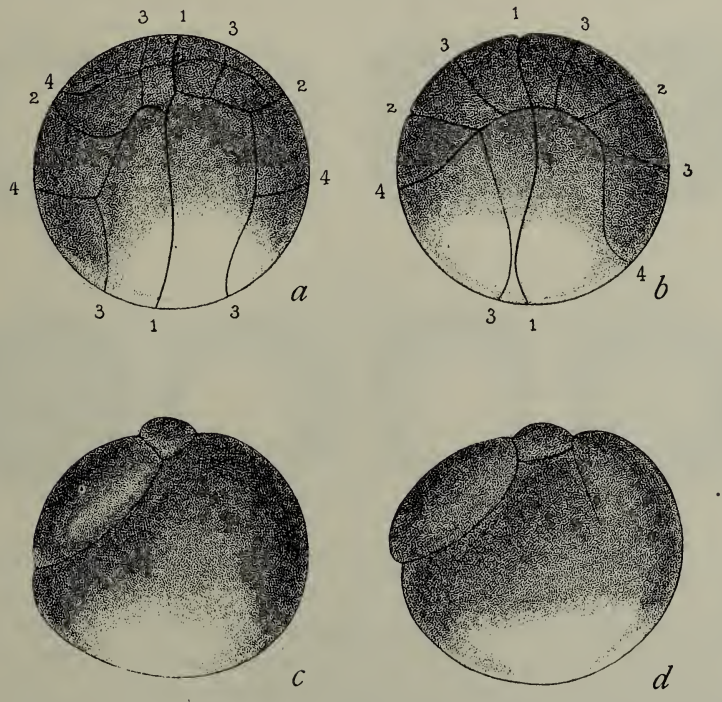


Fig. 205.—Cleavage of frog's egg compressed from the side. (After O. Hertwig.)

Roux ('85, '95) sucked up eggs surrounded by their jelly into tubes of smaller diameter than the egg itself, and observed that the first cleavage was generally at right angles to the long axis of the tube. Owing to the presence of the jelly, the shape of the eggs was often distorted and the cleavage irregular. Oscar Hertwig removed the jelly and sucked up the eggs into tubes whose diameter was less than that of the eggs. The eggs assumed a slightly oval or barrel shape with bulging ends. When the

eggs were drawn into the tubes from the side and the tubes kept in a horizontal position (with the black hemisphere above) the first cleavage was across the tube and through the pole, dividing the egg into equal halves (Fig. 206*a*). The second cleavage came in at right angles to the first and through the pole, i.e., in the long axis of the tube, hence in the normal position for the second cleavage (Fig. 206*b*). The third cleavage was not horizontal but parallel to the first and at right angles, therefore, to the tube (Figs. 206*c*, *d*). The fourth cleavages were horizontal. The loca-

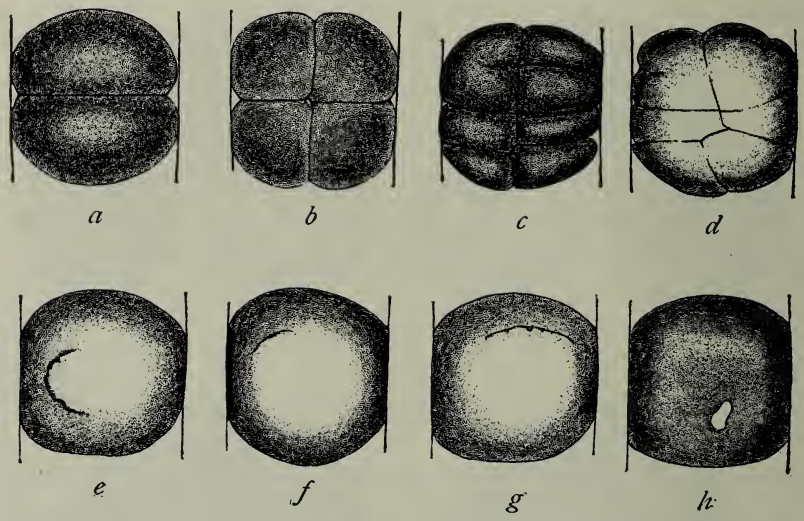


Fig. 206.—Cleavage and gastrulation of frog's egg drawn into a tube, and slightly elongated by pressure of walls of tube. (After O. Hertwig.)

tion of the dorsal lip of the blastopore is shown in Fig. 206*e*, *f*, *g*. It bears no obvious relation to the planes of cleavage. The position of the gray crescent was not recorded.

The Effect of Pressure on the Egg of Cumingia

The small eggs of the mollusc, Cumingia, are well suited for compression experiments, since the pigment bands make it easy to observe the orientation of the eggs (Fig. 207*a*). The large, polar spindle is present in the egg when laid, and if the egg is then fertilized, polar bodies are soon given off (Fig. 207*b*).

Browne ('10) has shown that if eggs are compressed as soon as laid, the two polar bodies appear at the pole (Figs. 207*c*, *d*, *e*).

When the egg is compressed in the direction of the egg axis, the polar bodies are still given off at the pole, despite the fact that the pole is now in contact with one of the compressing plates. The pressure does not change the direction of the spindle, nor prevent the polar body from being extruded from the surface. It is probable that the outer polar aster is already attached to or imbedded in the firmer surface layer of the egg, hence the spindle does not shift in response to the pressure.[2]

In the normal egg, the first cleavage passes through the pole, and presumably also through the point of entrance of the sperm, and divides the egg into unequal parts (Fig. 208*a*). In eggs

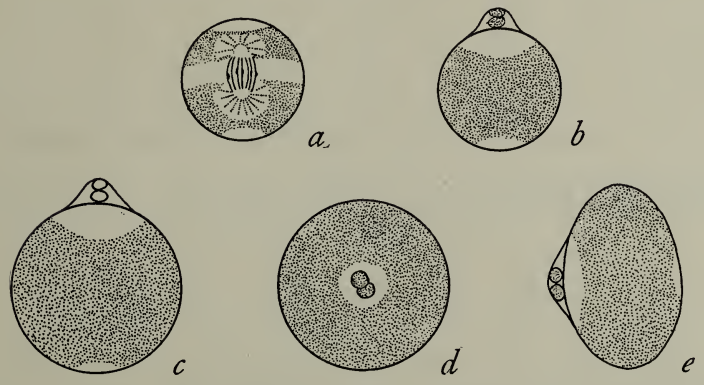


FIG. 207.—*a*, normal egg of Cumingia with polar spindle; *b*, egg after extrusion of both polar bodies; *c*, same under compression; *d*, same under compression from pole; *e*, side-view of last, after removal of pressure. (After Browne.)

compressed from the side, at any time before the first cleavage, the division in 55 per cent of the eggs is into equal parts (Fig. 208*d*, *e*, *f*); in the others it is into more or less unequal parts (Fig. 208*b*, *c*). Eggs that were much compressed gave 60 per cent of equal cleavages, those compressed least gave only 33 per cent. It is evident that the pressure may to some extent overcome the factors that give an unequal first cleavage. Presumably in the compressed egg, the first spindle takes a more nearly symmetrical position in the middle of the egg.

[2] King ('06) has studied the maturation of the eggs of Asterias under pressure. Most of the eggs produced abnormal polar spindles and one polar body, at most, was given off. The eggs were greatly compressed. The result was not due to absence of oxygen as control eggs in oxygen-free water gave off both polar bodies, although these were delayed.

When the egg is compressed from the pole, the first cleavage is more often into equal parts. In most cases the first cleavage of such eggs is through the pole, but not infrequently it lies some distance from the pole. It is not clear what causes this shift

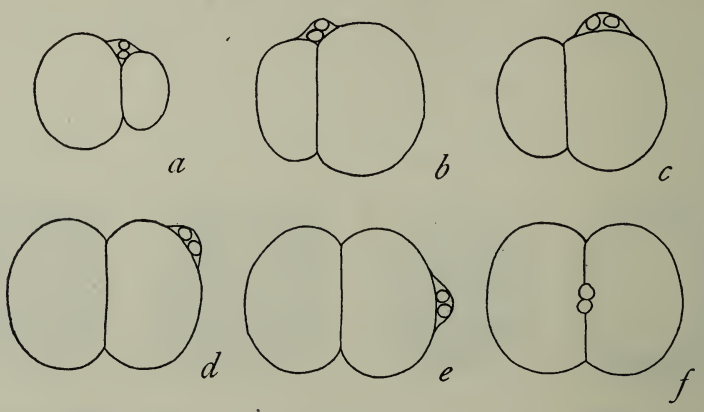


Fig. 208.—First cleavage of eggs of Cumingia under pressure. (After Browne.)

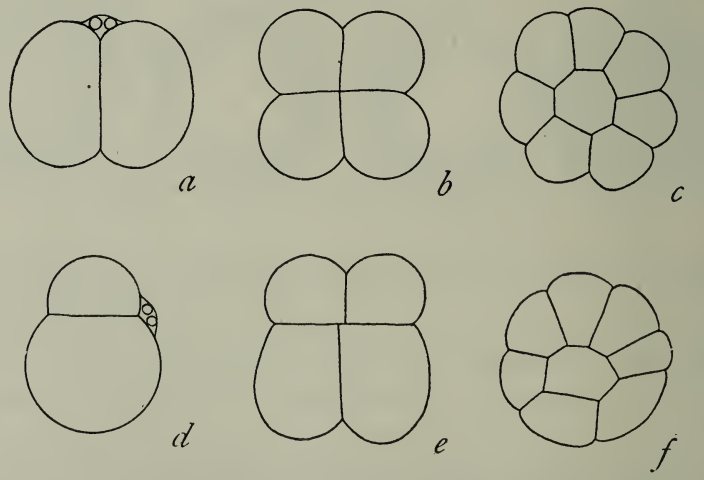


Fig. 209.—First and second cleavages of eggs of Cumingia under pressure. (After Browne.)

from the pole unless the position of the spindle is only in part determined by the pressure (lying therefore horizontally rather than obliquely) and being shifted to one side gives an unequal first cleavage as in the normal egg.

The relation of the point of entrance of the sperm in compressed eggs to the plane of division was studied by Miss Browne by watching the entrance of the sperm and then recording the cleavage of such eggs. No constant relation was found. The shifting of the spindle may be responsible for this lack of coincidence if the entrance point determines the cleavage plane in the normal egg.

The second cleavage of the normal egg divides the smaller cell into equal halves and the larger cell unequally. In compressed eggs, that have first divided equally, the second cleavages may come in at right angles to the first (Fig. 209*b*), or they may come in parallel to the first cleavage in one or in both cells. When the first division has been unequal (Fig. 209*d*) the second may divide each cell equally (Fig. 209*e*). The next division may likewise be into equal cells in both cases.

Embryos were not reared from such eggs, but since it has been shown (Morgan) that any slight disturbance of normal eggs of Cumingia prevents the normal development of embryos, any failure to develop, if found, might not be significant.

The Effect of Pressure on the Egg of Ciona

The cleavage of the egg and the formation of the embryo of Ciona are similar to those of Styela partita (see Figs. 28 and 126). Instead of the yellow pigment that makes the crescent so conspicuous a feature of Styela, there is in Ciona a pale gray substance that indicates the region of the crescent.

When the egg is removed from the oviduct the maturation spindle is present near the pole. If the egg is then fertilized, the movements of the ectosarc described for Styela occur in this egg also (Conklin). How far this movement is interfered with by pressure is not clearly shown by the experiments, because the movement is so rapid that one cannot be certain that it had not already taken place before the pressure was applied. Special experiments should be carried out with reference to this important point. The egg cannot be readily freed from its membrane, but can be compressed within it. The buckling of the membrane often brings local pressure to bear on the eggs causing them to assume curious shapes (Fig. 210*a*, *b*, *c*). Later, when the egg divides the membrane rounds out (Fig. 210*d*, *e*, *f*). The cleavages are

at right angles to the compressing plate (Fig. 210*e-i*). Eggs compressed during the time of the extrusion of the polar bodies, and set free before the cleavage spindle develops, may produce normal embryos provided the pressure period is not too prolonged (Morgan '10). If the spindle is kept under pressure long enough to be forced out of its normal position, there is the probability of abnormal development. For example, sixteen minutes after

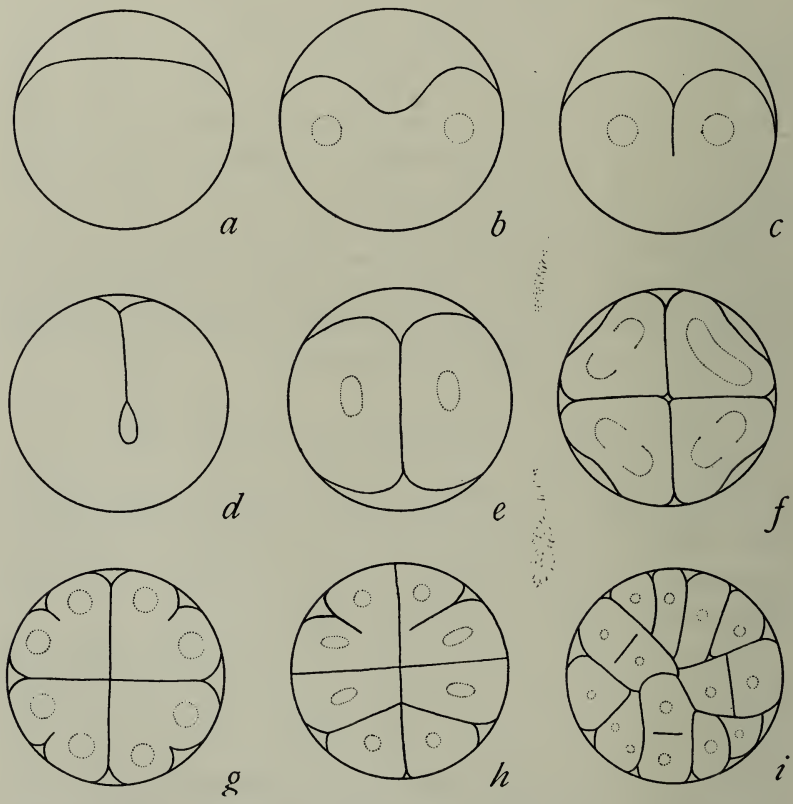


FIG. 210.—Eggs, and cleavage of eggs of Ciona under pressure. (After Morgan.)

fertilization, some eggs were put under pressure and kept there for fifteen minutes. They were then released (before the first cleavage had appeared). Later they segmented. Some of the embryos were normal, others abnormal. Other eggs of the same lot, thirty minutes after fertilization were compressed for ten to twenty minutes. Both normal and abnormal embryos were found

later. A third set was compressed ten minutes before division; when set free they were beginning to divide. More abnormal than normal embryos resulted. A fourth set was kept under pressure for twenty minutes before the first division, then released. Very few normal embryos resulted. In these four sets the proportion of abnormal embryos gradually increased.

Owing to the presence of the follicle cells outside the membrane, and the test cells within, it is difficult to identify the pole of the egg or the polar bodies when these eggs are compressed. How far the differences that are found within a set depend on how the pole lies in relation to the pressure, and how far due to the relation of the gray crescent to the cleavage planes, can not be stated. It appears, from a few observations only, that before the segmentation spindle begins to develop, the egg does not orient as the pressure is applied, but as soon as the spindle is formed, the eggs, when put under pressure, assume such a position that the spindle is parallel to the plates. This orientation takes place so promptly that it can not be due to the spindle moving into position as it lies in the protoplasm, but must be due to the egg as a whole shifting so that it takes a position with its sides to the plates. Such a result might be caused by the egg being more easily compressed in one plane than in another at this time, or because the egg has begun to elongate in the direction of the spindle. That the latter is the correct interpretation can scarcely be doubted, because as soon as the division plane is visible the shifting of the whole egg, as the pressure is applied, can be seen to take place. Moreover, the same conditions determine that, when pressure is applied at the 4-cell stage, the egg always assumes a position with its pole against one of the compressing plates. It also appears that when the 2-cell stage is compressed the egg is more often compressed from the "side" than from the pole. Here also the position assumed may be safely attributed to the shape of the egg rather than to the greater ease of compression in one plane than in the other, due to a difference in the rigidity of the contents in different axes.

If eggs have been kept for some time under pressure, their abnormal development might be supposed to be due to lack of oxygen. Special experiments were made to eliminate as far as possible this factor. Eggs in the early 2-cell stage were kept under pressure until they divided (four cells), when the pressure

was removed. Most of the embryos were abnormal. Other eggs were not put under pressure until just before a division. They were kept under pressure only during this division, and then released. The results were nevertheless the same as in the last case. Some other eggs, belonging to both these two series, were kept under pressure until the 8-cell stage. They too produced only abnormal embryos.

Eggs in the 4-cell stage were compressed until the eight cells had just appeared, others until the division had been finished. The divisions were at right angles to the plate and parallel to the first cleavage plane. Both sets gave abnormal embryos. Eggs in the 8-cell stage were compressed until the next division had taken place. All the embryos were abnormal. Eggs were compressed in the 4-cell stage and released at the 16-cell stage. All produced abnormal embryos.

The results of these experiments show that practically all of the eggs of Ciona, whose cleavage has been changed by pressure, produce abnormal embryos. The controls showed that these results were not due to the pressure as such, or to lack of oxygen, etc., but must have been due to direct effects brought about by shifting the plane of cleavage. In only a small percentage of cases could the egg have happened to be compressed in such a way that the first cleavage lay in the normal plane i.e., through the middle of the gray crescent. As the subsequent cleavages are influenced by the forced position of the first one (even after pressure has been released) such eggs would be cut up into cells whose boundaries do not correspond with the regional distribution of the materials that has taken place after fertilization.

The Effect of Pressure on the Egg of Crepidula

Conklin ('12) has subjected eggs of Crepidula to pressure. The presence of a jelly around the eggs, and their slow rate of cleavage make the experiment more difficult than in other cases. Irregularities in the formation of the polar bodies, and in the position of the first cleavage under pressure were observed, but the principal results relate to changes in the direction of the cleavage plane in eggs compressed after the first and second divisions have taken place. The third cleavage may be shifted, by compression in the primary axis, more or less into a meridional plane so that, instead of four micromeres being formed at the

next division, several or all of the resulting cells may be large. If the eggs are then released from pressure, those cells that contain part of the apical material may, at the following division, give rise to micromeres. If the pressure is applied after the first set of micromeres is formed so that the macromeres divide more equally at the fourth cleavage in a vertical plane, each of these macromeres in subsequent divisions gives rise to micromeres of the second and third sets. If the third cleavage is shifted to the equator at the 4-cell stage by lateral compression, each of the four cells at the pole produces in succession three micromeres, but no micromeres are produced from the four lower cells. In general, the results are similar to those seen in compressed eggs of other molluscs and annelids, and show that at the time of the third cleavage the micromere region of the egg is already determined.

The Effect of Pressure on the Egg of Nereis

The results of compressing the eggs of Nereis have been studied by Wilson ('96) and by Morgan ('10). Cleavage is at right angles to the compressing plates. The embryos that develop, after release from pressure, show many kinds of abnormalities, that are, however, often very slight.

Some eggs were compressed before the first division, others at the 2- and 4-cell stages. They were released at the 8-cell stage. All produced abnormal embryos.

If an egg in the 4-cell stage is put under pressure, it orients immediately, as the pressure is applied, so that the pole is against one of the compressing plates. The next division is at right angles to the plates, producing a group of eight cells in one plane. Each cell contains oil droplets that later run together (as in the normal embryo) and form a single drop in each cell. These eggs give rise to embryos abnormal in various ways. For instance, the eye-specks may be absent, or only present on one side, or even four eyes may be present (there are two eyes in the normal embryo). The setae may be suppressed, or appear only on one side. The pigmented trochal ring may be interrupted, or present only on one side. The distribution of the anal pigment may be abnormal. Wilson ('96) obtained a normal embryo from a plate of eight cells, but those seen by Morgan all showed abnormalities, although the latter were at times only very slight.

The Effect of Pressure on the Egg of Cerebratulus

Both Yatsu ('10) and Dederer ('10) have observed that, when the egg of Cerebratulus is compressed from the side before

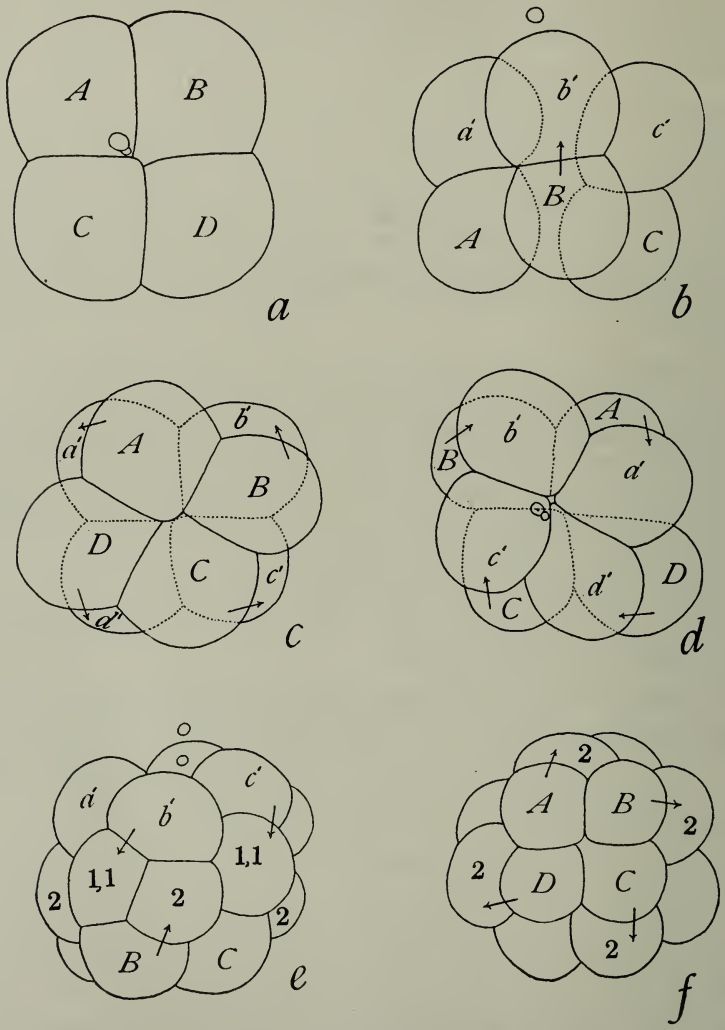


Fig. 211.—Normal cleavage stages of egg of Cerebratulus for comparison with cleavage of egg under pressure in Fig. 212. (After Dederer.)

cleavage, the first division is at right angles to the compressing plates and through the pole. The second cleavage is at right

angles to the first one, and also to the compressing plates. The third cleavage is also at right angles to the compressing plates.

Dederer has studied in detail the cleavage of the egg compressed from the side at the 2-celled stage. Such eggs are shown in Fig. 212*a-d* and for comparison the normal cleavage is shown in Fig. 211*a-f*. The same letters are used in both, but do not stand for the same cells. The cleavage of the compressed

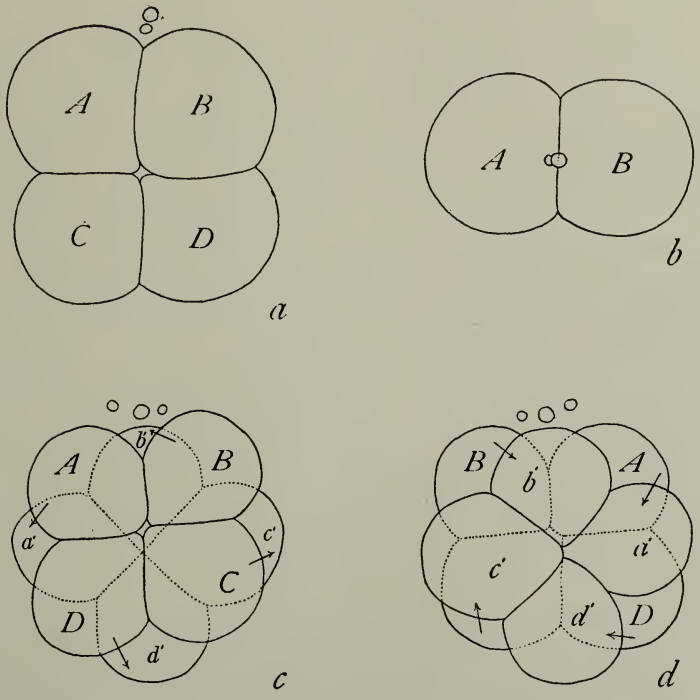


Fig. 212.—Cleavage of egg of Cerebratulus compressed from the side. Compare with Fig. 211. (After Dederer.)

egg (the second cleavage of the egg) is at right angles to the first (Fig. 212*a*, *b*). The two cells in the polar hemisphere (A, B) are in the majority of cases a little larger than the antipolar cells (D, C). It is interesting in this connection to note that in the normal cleavage, the four polar cells of the 8-cell stage are also larger than the four antipolar cells (Wilson). It seems not improbable that the results in both cases are due to the same internal condition.

The segmenting eggs were then released from pressure. The next cleavages (Fig. 212*c*, *d*) were nearly equal but showed a spiral arrangement. As a result, the 8-cell stage of the compressed egg, as seen from the side (Fig. 212*c*, *d*), is very similar to the normal 8-cell stage seen from the pole (Fig. 211*d*). Whether the factors that determine the normal, spiral type of cleavage determine the spiral arrangement of the compressed and freed egg is uncertain, but it does not appear improbable that they are the same. In both, the spiral is clockwise, which would seem to indicate some property of the cytoplasm then present, but not necessarily a spiral structure of the cytoplasm. It is possible, however, in both cases that the spiral arrangement represents only a secondary shifting of the eight cells into a more stable condition, the factor that determines the larger size of the polar cells being in some way responsible for the direction of the shift. Normal pilidia often result from eggs that have divided in this way.

Eggs, that had been kept under lateral pressure until an 8-cell plate had formed, and were then released, less frequently produced normal pilidia, although some of them were normal. Other eggs were more compressed, and the compression was begun immediately after fertilization and before the polar bodies were extruded. These eggs, kept under pressure until two divisions had taken place, either ceased to divide very far even when released, or if they cleaved they failed to develop into normal pilidia. It appears that the eggs are injured, if compressed at this time, or that some normal processes within them are interfered with.

Yatsu ('10) has described a modification of the cleavage of Cerebratulus that was produced in the following way: When the first cleavage appeared, the eggs were shaken to remove the membrane and transferred to calcium-free sea water. The second cleavage came in through the pole and at right angles to the first. The third cleavage was also meridional and through the pole, giving a ring of eight cells (or else a plate of a double row of eight cells). One may surmise that in the absence of the membrane and close contact, each blastomere became flattened, and divided at right angles to the substratum. The next (fourth) cleavage was equatorial or nearly so, giving eight large polar cells, and eight antipolar smaller cells arranged in two rings. The fourth cleavage here produces the same pattern as does the third

cleavage of the normal egg. In other words: a third cleavage has been interpolated which doubles the number of "micromeres." [3]

How Does Pressure Determine the Direction of the Cleavage Planes?

The experiments show quite definitely that the position of the cleavage plane of the compressed egg is determined by the position of the spindle at the time of cleavage. The division takes place at right angles to the spindle and through its equator. The position assumed by the spindle is evidently related in some way to the direction of the pressure. If the causes that lead to the position taken by the spindle in the compressed egg could be ascertained, the principal mechanical problem involved in the experiments would be answered. Several attempts to solve the problem have been made.

Pflüger suggested that the spindle develops in the direction of least resistance, which he assumed would be at right angles to the direction of the pressure. Before such a view can be accepted, it would be necessary to show that the egg is a semi-solid body in which pressure lines can be produced; but, if the egg is only a fluid enclosed in an elastic wall, the pressure will be equal in all directions, and pressure lines cannot be invoked. Even if the egg is regarded as a semi-solid body, it is gratuitous to assume that the spindle would develop in any particular relation to the lines of pressure, for we know little as to the nature of the spindle.

It has been suggested by Oscar Hertwig ('93) that the spindles develop in the direction of the greatest protoplasmic mass. This statement is sometimes spoken of as a law, but it should not pass unnoticed that it is little more than a re-statement of what is found in certain cases and that it does not seem to apply in other cases. There are also other results that appear to contradict

[3] The results of compression of a few eggs of Beroë have been described by Ziegler ('98). One egg about to divide was compressed from the side. The next division into four was brought about by two divisions vertical to the compressing plates and parallel to the first division. At the next division the plane of cleavage also started at right angles to the plates, but the cells twisted somewhat out of position as the cleavage advanced.

such a "law." A few illustrations will suffice to show the insufficiency of such a rule to explain the phenomena.

When the first polar spindle is formed it usually lies deep in the egg, often near or just above the center. It then moves to the pole where division takes place. Since the polar spindle is in all respects similar to spindles in ordinary cell-divisions, it is evident that the position it takes at the pole of the egg cannot be explained by the distribution of the greatest protoplasmic mass at the time of its migration.

The segmentation spindle usually lies in a plane at right angles to the primary axis and as a rule near the center of the egg. It lies nearer to the pole when much yolk is present. In both cases it cannot be said to extend in the direction of the greatest protoplasmic mass, since there is not one, but an infinitive number of possible lines in this plane, all of which conform to this requirement. The particular one that it takes has been shown in several cases to be due to other factors. Hence the explanation based on protoplasmic mass alone takes into account only one of the conditions that determine the position of the spindle.

In the 4-cell stage of the frog's egg, and of several eggs like it, the third division is equatorial, which calls for a "vertical" spindle. It has not been shown that a vertical line is more nearly in the direction of the greatest protoplasmic mass when yolk is present, than a horizontal line would be. In fact, the spindle must to some extent move down into the yolk hemisphere in order that the division be near the equator of the egg. In order to do this some of the yolk must first be moved aside, and this is in fact what happens.

When the egg of the sea-urchin is fertilized simultaneously by two spermatozoa, a triaster with three poles often develops. The triaster lies in a "horizontal" position, i.e., at right angles to the primary axis. The first division of the egg is, then, into three parts. The next division is again through the pole, dividing the egg into six cells in one plane (Fig. 10). It is not apparent that the greatest protoplasmic mass is such that these second spindles should be in a horizontal position, but it is apparent that the second division is in the same direction as it is in the normal egg of the same stage. At the next division the spindles are vertical, as in the normal, and may then be said to lie in the greatest protoplasmic direction. But at the next division

six of the cells form micromeres in the egg with threefold cleavage, while in the "lower" hemisphere the six cells divide meridionally. In both instances the division is obviously the same as that of the corresponding stage of the normal egg, but the difference in the amount or distribution of the protoplasmic masses in the cells of the two hemispheres does not seem a sufficient explanation of the position taken by the spindles in them. The same statement also applies to the corresponding division of the normal egg. It is true one may imagine that a redistribution of the protoplasmic and yolk masses takes place after each division of such a kind as to place the protoplasmic masses so that the long axis anticipates the next position of the spindle, but there is no evidence at present that such a change takes place, and if this happens it is the shifting itself that calls for explanation.

In some dispermic sea-urchin eggs the first cleavage divides the egg into four equal cells around the primary axis (Fig. 11). Each quadrant is longer in the direction of the polar axis, yet at the next divison each quadrant divides by a plane passing through the pole and the primary axis. Here again the position of the spindle is not in the direction of the greatest length of the blastomere, but at right angles to it, etc.

This and other evidence make it probable that other factors than the one appealed to by Hertwig determine the position of the spindle in the cell.

In centrifuged eggs the maturation spindle of the egg of Cumingia moves to the pole irrespective of the kind of materials (inclusions) that have been driven into the polar field, and since this region may sometimes contain most of the yolk of the egg, it is evident that yolk will not prevent the spindle from taking its normal position. In the egg of Cumingia, the first division is into very unequal cells, even in the centrifuged eggs. In some cases the spindle must move into the yolk field, that may have been filled with yolk by long centrifuging, yet the size of the two cells is the same as in the normal egg. Here again the spindle must develop and move into its final position irrespective of the yolk already in the path of its future development.

In these cases it may be necessary to distinguish between the yolk and the substratum of the egg into which the yolk is thrown. This substratum probably remains to some extent in position after the yolk has been driven into it. If the growth of the spindle

is determined by this substratum, rather than by its inclusions, the extension of the spindle may be still determined by the same material as in the normal egg. However this may be, the results suffice to show that it is doubtful how far the yolk, or other like inclusions, can be used in an argument as to where the so-called greatest protoplasmic mass lies. If this is admitted it becomes apparent that there is no objective way to find out where the greatest protoplasmic mass is situated, hence, the uncertainty of making it responsible as the causal agent in directing the position of the spindles.

As the spindle develops there is formed about it a hardening of the surrounding protoplasm (gel). The result is that an elongated semi-solid spindle with rounded ends lies floating in the more fluid surrounding medium. There is some evidence, but not enough at present, indicating that the spindle, when it is formed, may be shifted and come to lie at right angles to the compressing plates. This would seem to be a position of greater equilibrium; but at present we do not know enough about the conditions in the egg and their possible influence on the direction and rate of growth of the spindle to permit us to do anything more than speculate as to how such conditions affect the formation of the spindle.

General Bearing of the Results on Problems of Development

It is obvious from the results of the compression experiments that, while normal development may take place in the eggs of hydroids, sea-urchins, frogs, and Cerebratulus after cleavage under pressure, yet in the case of Nereis, Ciona and probably in molluscs, abnormal development follows compression. Several possibilities suggest themselves. In the first group a relatively larger number of cells is present when the differentiation of the embryo begins than in the latter group where fewer cells are present. It might appear at first sight that the differences in the two cases are connected with the relative number of cells present before they become differentiated. If differentiation sets in when only a few cells are present it is possible that the boundaries between the cells in the compressed eggs may not then correspond with the regional differences that were earlier initiated.

Hence, the different organs that develop will have abnormal size-relations and positions in comparison with those of the normal embryo. In the ascidian, for example, the development of the organs is largely dependent on the coming together of cells on each side of the blastopore. Irregularities in the sizes and positions of the blastomeres might be supposed to interfere with this union, hence, in part, the failure to develop normal embryos from compressed eggs of this type. The appeal here is not so much to the number of cells present before differentiation as to the time at which a visible differentiation can be detected. If we turn then, to this side of the problem, certain facts may be brought into a more or less plausible connection with the evidence. In eggs like those of the sea-urchin, of Amphioxus, and of the frog, an irreversible differentiation of the blastomere seems to take place relatively later in development than in the eggs of molluscs, annelids and ascidians, hence changes in the direction of the cleavage planes cause fewer mechanical difficulties to development in the former than the latter. It is, however, by no means to be taken for granted, as here implied, that the differentiation referred to occurs after the cleavage of the blastomeres has taken place. It may very well be that the initial steps are taken during the division. Moreover, the essential problem may lead back to the egg itself. Therefore, while it still remains possible that departures from the normal size-relations may affect more seriously the mechanical problem in eggs that become differentiated when fewer cells are present than when a larger number of relatively smaller ones are present, there may still be more fundamental factors involved in the situation.

The experiments with compressed eggs have furnished evidence that has been used both by Driesch and by Oscar Hertwig as a strong argument against the mosaic theory of development held at one time by Roux and later developed by Weismann. Pflüger, at a still earlier date, had reached conclusions diametrically the opposite of that of Roux and Weismann. In so far as the mosaic is supposed to depend on a qualitative sorting out, during cleavage, of the determinants of the chromosomes, the experiments on compression can still be used to show the improbability of such a view, at least in those cases, which are not numerous, where normal development takes place after compression. On the other hand, if a mosaic theory of development that

appeals to cytoplasmic regional differences in the egg is in question, the results of compression might be utilized in support of such a view. But even then there would remain the two kinds of apparently contradictory results, since changes in the cleavage do not produce abnormalities in certain eggs, and do produce abnormal development in other eggs.

CHAPTER XXII

THE REDISTRIBUTION OF THE VISIBLE MATERIALS OF THE EGG BY CENTRIFUGING

The centrifuge has proven an instrument of extraordinary delicacy in bringing about a new distribution of the formed materials of the egg, such as the yolk, the pigment, and the fats. The forced transfer of the more solid or semi-solid constituents (the inclusions or "formed materials," so-called) through the egg, does not seem in any way to injure its living substance. By the use of the centrifuge it is possible to study many problems connected with the possible rôle of these substances that have sometimes been looked upon as formative-substances or organ-forming materials, i.e., materials whose presence in excess in any cell was supposed to determine its future course of development. It may be stated at the outset, that the results with the centrifuge have shown very convincingly that these substances, as far as examined, have no such function in development. It does not follow, of course, that there may not be other substances that do have a determinative influence on development. In the earlier experiments in which the eggs of the frog were centrifuged, the speed of rotation was not high enough to bring about a quick separation of the materials of the egg. The method was used with other ends in view, namely, to determine whether gravity has an influence on development, or else to bring about differential injury to parts of the egg (Pflüger '83; Roux '84, '85, '87, '03; Schultze '87, '94; O. Hertwig '94, '98; Kathariner '01, '02; Moszkowski '02; Morgan '02, '04; Wetzel '04).

An experiment utilizing a rate sufficiently high to bring about a redistribution of the contents of the egg was first made by Gurwitsch ('04), who employed the centrifuge to study the constitution of the cytoplasm. He concluded that since the yolk granules can be driven through the egg of Triton and frog without destroying its capacity to develop, the protoplasm has no permanent structural basis, but is essentially a fluid.

Two years later Lyon ('06) centrifuged, at a high rate of speed, the eggs of the sea-urchin, Arbacia. The materials were separated into four well-marked zones (Frontispiece). Lyon also made the significant discovery that the new distribution of the constituents of the egg bears no determinative relation to the parts of the embryo that develop from the centrifuged eggs—a conclusion that has later been extended to practically all other eggs that have been studied by centrifuging.

The Development of Centrifuged Eggs of the Sea-Urchin

(*See Frontispiece*)

As Lyon ('06) first showed, it is possible, with a small hand centrifuge, whose arms revolve at the rate of about 10,000 revolutions per minute, to separate in five minutes or less the materials

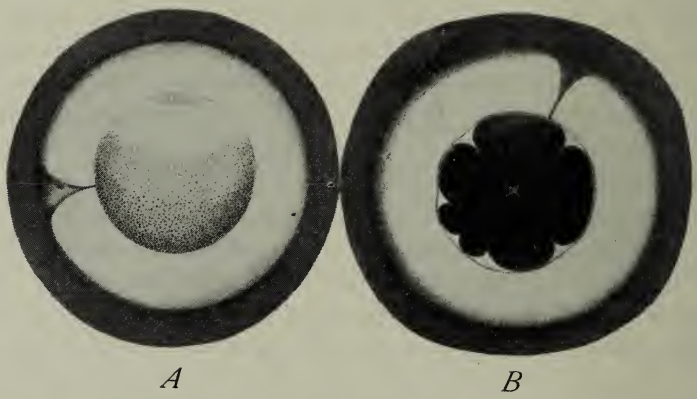


Fig. 213.—Centrifuged eggs of the sea-urchin, Arbacia. *A*, stratified egg of Arbacia surrounded by the clear jelly, the funnel lies at one side; *B*, an eight-cell stage with micromeres opposite funnel. (After Morgan and Spooner.)

of the egg of the sea-urchin into four zones (Fig. 213*A*). The lightest substance of the egg, which comes to lie at the side nearest the center of rotation (the inner or centripetal pole), is the fat, sometimes spoken of as the oil. Next to this is a "clear zone" containing much of the more watery material of the cytoplasm. Beyond this is the yolk, and around the centrifugal or outer pole is the pigment. The two latter substances are not completely separated, the pigment lying, for the most part, more superficially than the yolk, and around the "outer" pole. The nucleus of the

egg is carried into the clear protoplasmic zone, and lies just beneath the layer of fat.

The three principal substances, fat, yolk, pigment, composed respectively of droplets, spherules, and more solid particles, do not fuse (or only in part) when they are forced to come together in special zones, but remain as separate bodies, owing, probably, to a covering layer of protein, or other material. It may be that there is "cytoplasm," sometimes spoken of as the ground-substance, occupying the space between the individual spherules. When removed from the tube, the stratification of the egg persists for a long time, even throughout the cleavage.

If the eggs are fertilized first, and after several minutes are then centrifuged until well stratified, they begin to segment at the same time as the control, normal eggs. The first cleavage (Fig. 214*a*; see also the Frontispiece) is at right angles to the stratification (Lyon '06, Morgan and Lyon '07). The cleavage plane passes through the fat pole, and, then, through the other layers, dividing the substances into identical halves. The second cleavage is parallel to the stratification (Fig. 214*b*), dividing the first formed cells into equal or nearly equal halves; one half, however, containing the fat and most of the clear zone, the other half containing some of the clear zone, most of the yolk, and nearly all of the pigment. The third cleavage is at right angles to the two preceding planes of division producing eight cells equal in size. This cleavage is in the plane of the paper, and is not shown in these figures.

At the fourth cleavage, the four micromeres appear at one pole of the egg around the intersection point of two of the preceding cleavages. The two intersecting planes may be either the first and third, in which case the micromeres will lie either in the oil zone (Fig. 214*c*) or in the pigment (Fig. 214*d*), or they may lie at the intersection of the first and second planes, and, if so, the micromeres will lie near the line between the clear zone and the yolk (Fig. 214*c'*). At whatever point the micromeres appear they are about the same size, and are relatively clear cells free from the material above which they lie. It appears that their cytoplasm is derived from some interior material that comes to surround the outer pole of the spindle, which, pushing the surface of the egg to the sides, is constricted off to form the micromeres.

At this same (fourth) cleavage of the centrifuged eggs, the four cells of the hemisphere opposite to the micromeres divide by meridional cleavages into equal parts (Fig. 214*c*, *d*, *c'*). The cleavage pattern is identical with that of the normal 16-cell stage.

It has been shown (Morgan and Spooner, '09) that the location of the micromeres in the centrifuged egg is largely determined by the original axis of the egg. They lie, as nearly as is consistent with the meeting point of two cleavage planes, at the antipole of the egg, as they do in the normal cleavage. The evidence for this conclusion was obtained by locating the micromeres of centrifuged eggs in relation to the funnel in the jelly (Fig. 213*B*).

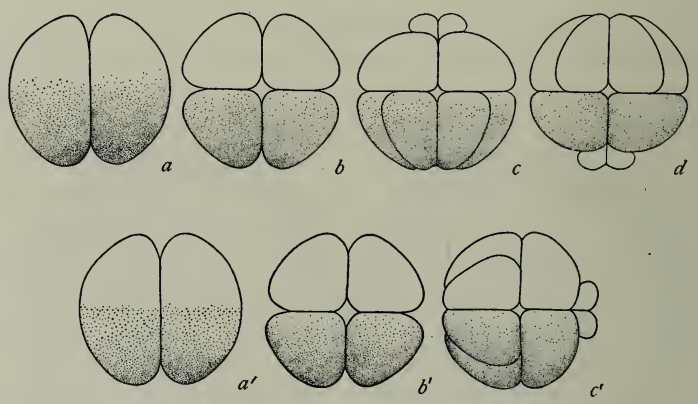


FIG. 214.—Cleavage of centrifuged eggs of Arbacia in which the first plane of division is at right angles to the stratification, and the second at right angles to the first and parallel to the stratification. The third cleavage is in the plane of the paper. The fourth cleavage is shown in *c*, *d* and *c'*; the micromeres may lie at the centripetal pole (*c*), at the centrifugal pole (*d*), or at the side (*c'*). (After Morgan.)

For example, if the centrifuged eggs after fertilization are put into a thick solution of India ink, rubbed up in sea water, the funnel soon becomes apparent. The stratification is then seen to have taken place without regard to the primary axis of the egg. This means that the eggs have fallen at random in the tube, when the centrifuging began, and they have been stratified without changing their position. Other evidence substantiates this conclusion. The micromeres are found to lie opposite to the funnel in the jelly. It follows that while the stratification affects the location of the first three cleavages, the fourth cleavage is not affected (or only slightly so) either by the stratification or by the preceding cleavages.

In normal eggs, the position of the first cleavage is determined by the position of the mitotic figure. In the centrifuged egg, the spindle, as it develops, comes to lie within the clear zone just above the yolk in a position parallel to the stratification. At first sight it may seem probable that its position is due to the greater density of the yolk hemisphere that would interfere with the extension of the spindle into the yolk mass, which would have to be displaced if the spindle occupied any position other than one parallel to the yolk zone. It may appear plausible that the position of least resistance for the elongation of the mitotic spindle is in such a "horizontal" plane. On the other hand, the

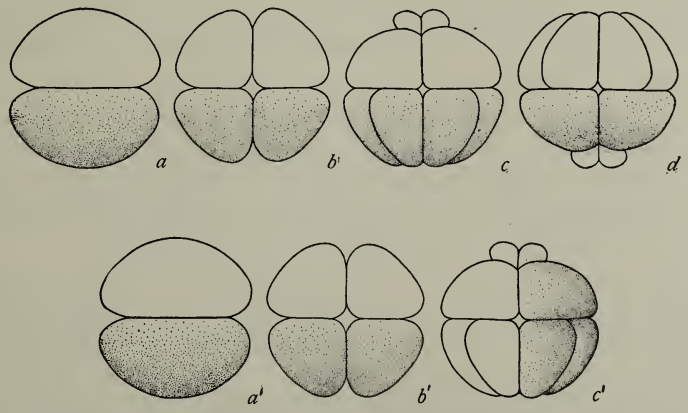


Fig. 215.—Cleavage of centrifuged eggs of Arbacia in which the first plane of division is parallel to the stratification; the second at right angles to the first and to the stratification; the third in the plane of the paper. The fourth cleavages, *c*, *d*, *c'*, may have the same position as in Fig. 214. (After Morgan.)

cytoplasmic substance of the clear zone may be more suitable for the growth of the asters—hence they extend out into it. But both of these conclusions seem to be contradicted by the position of the second spindles, since these spindles lie parallel to the first division plane; hence one end of each must penetrate some distance into the yolk-zone. In order to do this, the spindle must extend almost as far into the yolk as would the first spindle if it developed at right angles to the stratification. Fortunately, no appeal can be made in this case to any original "direction" present in the egg-substance as a factor in placing the spindle—an appeal that has been frequently made to account for the position assumed by

cleavage spindles—because the evidence shows beyond a doubt in the centrifuged egg that the position of the first three spindles, at least, bears no fixed relation to such an imaginary directing agent, but is determined by the stratification.

That a mitotic spindle may, after it forms, be altered by the centrifugal force can readily be shown. Spooner ('11) who examined into this question, found that if the eggs are centrifuged 40 minutes after fertilization (when the first cleavage spindle is fully developed in the eggs), the first cleavage appears at right angles to the stratification in most of the eggs. Sections of these eggs show a normal mitotic spindle, not in the middle of the egg, but under the oil cap in the clear zone. The evidence proves that the spindle is carried into a position parallel to the stratification, or else the eggs orient at this stage as they fall in the centrifuge. Just how the spindle gets into this position is not clear, but if, as has recently been shown, the two asters around the poles of the spindle are centers of solidification (gel), and if this material has a specific gravity greater than the oil, and is only a little lighter than the clear zone, then the only position of equilibrium for such a spindle is one parallel to the stratification.

If the eggs are *first centrifuged and then fertilized*, the first cleavage generally lies as in the preceding case, at right angles to the stratification, but in a few cases the first cleavage is parallel to the stratification (215*a*, *a'*). A study of the fertilized eggs shows that the spermatozoön may enter at any point of the surface, and that the sperm-nucleus may, therefore, meet the egg-nucleus at any angle. In other words, its movement towards the egg-nucleus is little affected by the stratification. If, then, the position of the spindle, as it develops, is little, or not at all, affected by the stratification, the cleavage may be expected to make any angle with the layers, and this is to some extent true, although it is as a rule at right angles to the stratification. As stated above, the first division of a few eggs is in a plane parallel to the stratification. This result shows that the elongated spindle finds no insuperable difficulty in pushing into the yolk layers. In these cases the position of the spindle is probably determined by the plane of apposition to the two pronuclei. The latter is unaffected by the centrifuging, that had already taken place, and not much affected by the stratification. Three typical positions of the two nuclei are shown in Fig. 216.

The occasional occurrence of eggs dividing parallel to the stratification, even in sets of eggs centrifuged after insemination, may be explained as due to late fertilization; for if an egg should fail at first to be fertilized, and is then centrifuged, and later a sperm enters (Fig. 216*a*, *c*), it may sometimes cleave, at first parallel to the stratification.

The fourth cleavage is the first "differential" cleavage, by which is meant no more than that an unequal division takes place in one hemisphere that produces the micromeres. The most striking fact in regard to the micromeres of the centrifuged egg is that they are formed in entire disregard of the kinds of material that the centrifuge has thrown into that region of the egg at which they are destined to appear; namely, the region opposite the funnel. It may, therefore, appear that their material is

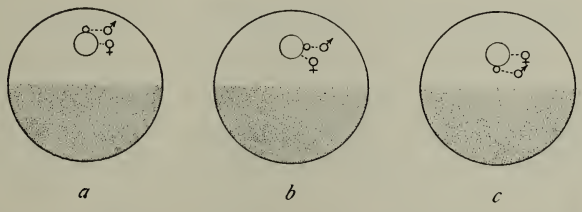


FIG. 216.—Diagrams showing possible positions of the two conjugating nuclei in eggs centrifuged before fertilization, and fertilized afterwards. (After Spooner.)

derived from some central material that is not much affected by the centrifuging, or else arises during the first two cleavages. It does not seem probable that their position is determined by some sort of polarity of the materials of the whole "germinal substance," because the regional differences may be almost entirely changed by driving the oil, or the yolk, or the pigment into the polar field.

Lyon showed that gastrulation takes place without regard to the stratification produced by the centrifuge. Morgan showed that the micromeres in the centrifuged egg form opposite the funnel in the jelly as in the normal egg. Spooner then showed that the micromere region of the centrifuged egg is the region at which gastrulation takes place, thus completing the chain of evidence proving that gastrulation is at the micromere pole in the centrifuged eggs, and hence is independent of the induced redistribution of the visible substances of the egg.

As Lyon first showed, the embryo (pluteus) from centrifuged eggs develops without regard to the artificial distribution of the movable materials of the egg. These plutei are normal in form and function. The conclusion is obvious that neither the fat, nor the yolk, nor the pigment granules are organ-determining materials. That the two former serve in one way or another as materials for later development can scarcely be doubted, but that they do not determine the fate of the cells in which they are contained is demonstrated by these results. It is true that the plutei have not been reared to adult sea-urchins, but since in two other animals (rotifers and copepods) centrifuged eggs have been kept until the adult stages, which were normal, were reached, it is practically certain that normal sea-urchins would also develop from centrifuged eggs.

The chemical composition of the eggs of the sea-urchin Arbacia has been studied by McClendon ('09). Mature ovaries, with ripe eggs, were freed from the body fluids, frozen, and ground up in a mortar at a low temperature. The material was strained through bolting cloth to remove the ovarian stroma and transferred to a centrifuge making 3200 revolutions per minute at a radius of 154 mm., and rotated eight hours or more. The materials separated into two layers, a jelly-like centrifugal layer corresponding to the yolk-half of the egg, along with the jelly of the egg which greatly increases its volume, and a centripetal layer corresponding to the clear middle layer of the egg. The fat did not separate as a layer. The materials were weighed, rapidly desiccated, and weighed again to get the water-content, etc. The ether extract of the centripetal layer was found to contain "myelin forms," probably lecithin mixed with red pigment, and crystals resembling fat crystals. The extract of the centrifugal layer gave similar results except that fewer fat crystals were present. Comparing these and other results of chemical analysis of the Arbacia egg with those of the frog, McClendon concludes that while there is quite a difference in the water content in the layers of the centrifuged frog's eggs, there is very little difference in the Arbacia egg. While there is a great difference in the relative amounts of extracts and residue in the layers of the frog's egg, in Arbacia a great difference is seen only in the water extract which is largely composed of salts, etc. McClendon suggests that the greater

injury produced by centrifuging the frog's egg, than by centrifuging the Arbacia egg, may be due to the fact that fewer differences are produced in the latter than in the former. It is equally probable, I think, that in so far as the results are different they can be accounted for in other ways. The most important fact is that both kinds of eggs may be well centrifuged, and produce normal embryos.

Centrifuging the Eggs of Cumingia

The eggs of the little bivalve mollusc, Cumingia, lend themselves admirably to experiments with the centrifuge. The females, if kept dry for a short time after being dug up from the mud

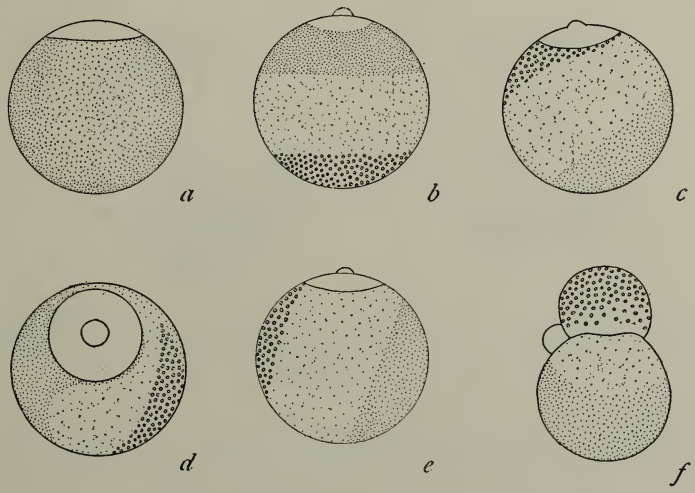


Fig. 217.—Eggs of Cumingia that had been centrifuged before fertilization: *a*, normal egg; *b*, centrifuged egg giving off first polar body in fat zone; *c*, same in yolk zone; *d* and *e*, same in clear middle zone; *f*, first cleavage. (After Morgan.)

in which they live, will set free their ripe eggs soon after they are placed in dishes of sea water. The spindle for the first polar body is already present in the egg at this time. It is relatively large, occupying almost the entire egg; its rays reach almost or quite to the outer membrane. After centrifuging, the egg shows four zones (Fig. 217*b-e*), similar to those in centrifuged eggs of the sea-urchin. There is (1) a large oil cap; (2) a broad,

clear zone; (3) a small yolk field; and (4) a pink, pigmented region opposite to the oil cap. The position of the spindle is not affected by the centrifuging, and since the eggs fall at random, the spindle may lie in any possible position with respect to the stratification.

As soon as the centrifuged egg is fertilized, the polar spindle moves towards the surface, becoming smaller as it advances (Fig. 218*a*). A minute polar body is given off (Fig. 217*b*, *c*). It may lie in the pigmented region, or to one side in the yolk, or in the fat, or at the edge of the clear zone. Its interior contains little or none of the materials through which the end of the spindle has advanced to reach the surface. There can be no doubt that the first polar body has been formed at a predetermined pole, and

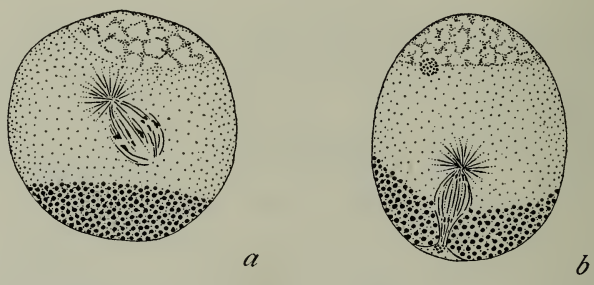


FIG. 218.—Two sections of centrifuged eggs of Cumingia. In *a*, the segmentation spindle is in the middle of the egg; in *b*, the spindle has moved into the yolk field. (After Morgan.)

its location is unaffected by the centrifuging. The second polar body forms soon afterwards at the same point. The two pronuclei then unite in the interior of the egg, and the segmentation spindle develops. If the centrifuging takes place at this time rather than before, there is no evidence that the position of the segmentation spindle can be changed.

The first cleavage divides the egg unequally (Fig. 217*f*; 219*a*, *b*). It always passes through the pole, and then turns to one side to cut off the smaller cell. This smaller cell may contain the fat cap, or may be composed entirely of yolk or of pigment, or it may be made up largely of the material of the clear zone. Its content, and therefore presumably its location, are unaffected by the material that has been thrown into the region through which the cleavage furrow is destined to pass. What factor determines

that side of the egg through which the first cleavage plane cuts, is not known, but from a comparison with the results on the egg of Nereis, that has a similar type of cleavage, it is not improbable that the path of entrance of the sperm nucleus (the copulation path) fixes this relation by determining the location of the spindle.

At the second cleavage the large cell divides unequally, and the small cell divides into equal parts. This division occurs with the same precision in all eggs without respect to the distribution

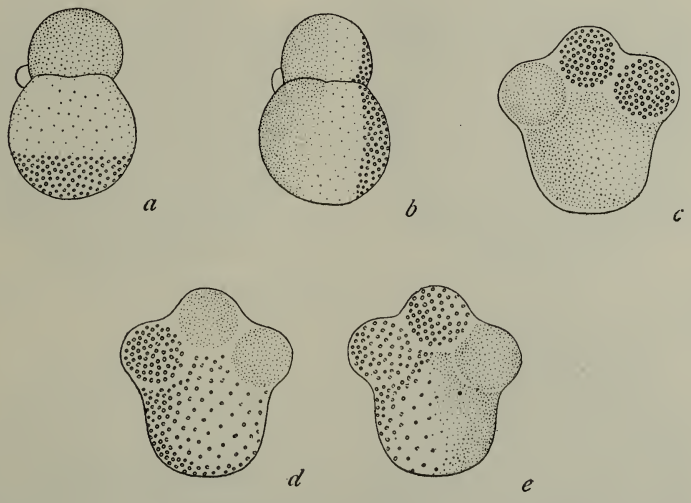


FIG. 219.—Cleavage of centrifuged eggs of Cumingia: *a* and *b*, first cleavage, *c*, *d*, *e*, second cleavage. (After Morgan.)

of the materials contained in the different regions (Fig. 219*c*, *d*, *e*).

At the next division, the first quartet of micromeres is formed around the pole. From these eggs, trochophore larvae develop (Fig. 220*a*, *b*, *c*), provided the eggs have been handled with extreme care. If not treated very gently, the cleavage may still be entirely normal, but the embryo abnormal.

The best way to insure success is to centrifuge the entire animal on a water centrifuge in large tubes. If centrifuged for twenty minutes, the females will lay their eggs soon after removal. These eggs may be completely stratified. If fertilized, they will give normal embryos.

The normal development of the stratified eggs of Cumingia

furnishes positive proof that the visible materials of the egg are not formative materials in the historical use of this term, and this is significant, for, the Cumingia egg divides in the same way

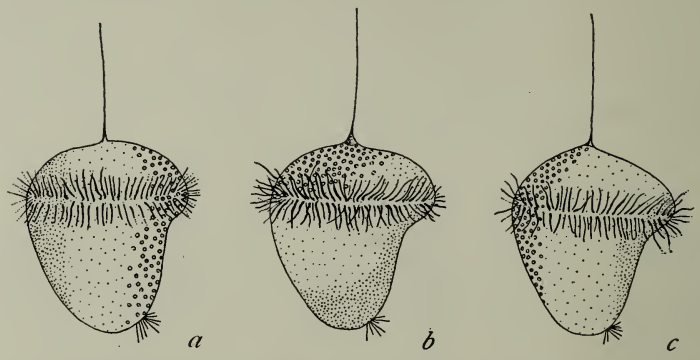


FIG. 220.—Three trochophore larvae of Cumingia from centrifuged eggs. The yolk and fat-fields lie in different positions. (After Morgan.)

as do eggs with a strictly determinative type of cleavage and early differentiation.

CENTRIFUGING THE EGGS OF CHAETOPTERUS

The eggs of the marine annelid, Chaetopterus, have been frequently used in experimental work. The ripe egg, with large ovarian nucleus, is easily obtained by snipping off portions of the parapodia of the female that are filled with eggs. Sperm can be obtained from the male in the same way.

The ovarian egg is attached to the wall of the ovary. The free end of the egg becomes the pole, the attached end the antipole. When set free, the egg has the following structure (Figs. 26*b*, *c*, and 221*a*). According to Lillie ('06, '09), an ectoplasm covers the polar hemisphere extending a short distance below the equator. At the pole there is a defect in the ectoplasm that allows the endoplasm to come to the surface. The polar bodies are later extruded from the middle of this region. Embedded in the ectoplasm are from one to three layers of clear colorless spherules. They stain differently from the spherules in the interior. The endoplasm of the polar hemisphere contains large yellowish spherules that stain black in osmic acid. There are fewer of them in the protoplasm just above the nucleus. In the other

hemisphere the protoplasm is vacuolated and contains smaller spherules that stain differently from the larger ones in the polar half of the egg. The endoplasm comes to the surface over the lower hemisphere. The large nucleus of the egg has about one-

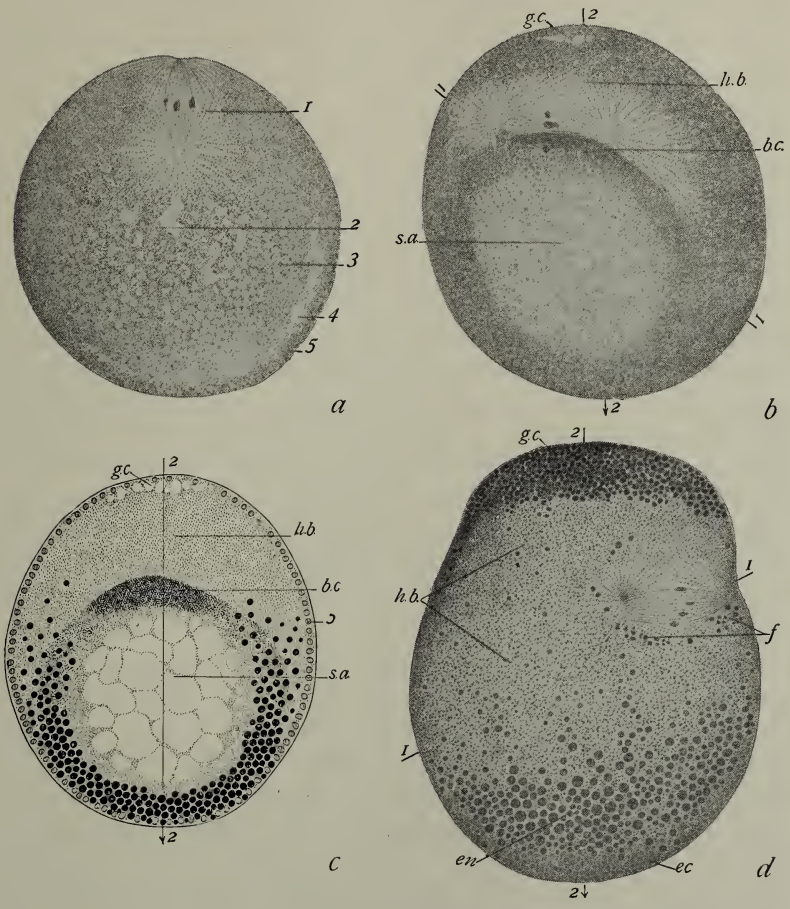


FIG. 221.—*a*, section of egg of Chaetopterus with polar spindle; *b*, partially centrifuged egg; *c*, section of more completely centrifuged egg; *d*, very completely centrifuged egg, preserved so that the material of the fat cap is retained. (After Lillie.)

eighth the volume of the whole egg (Fig. 26*a*). It contains a large nucleolus to which some at least of the chromosomes are attached. The formation of the maturation spindle, which develops soon after the eggs have been exposed to sea water, has been

studied by Mead and by Lillie. At the time when the nuclear wall is about to disappear, a number of centrioles with a few rays appear at numerous points in the protoplasm about the nucleus (Mead). Two of these become the poles of the maturation spindle to which the chromosomes become attached when the nuclear wall disappears (Fig. 26*b*). The spindle occupies a large part of the polar hemisphere. As soon as the nucleus breaks down the ectoplasm begins to extend over the antipole, and has completely covered it before the maturation spindle has become "fixed" to the pole. This movement, as seen in the living egg, appears to consist of, or to be accompanied by, a wave-like motion in the layer outside the ectoplasm (the pellicle). The protrusion of endoplasm at the pole becomes enlarged at this time.

In the endoplasm extensive changes take place. The protoplasm beneath the pole, with its large spherules, flows outward and around the nucleus toward the antipolar hemisphere, where it mixes with the material of that hemisphere. The less granular material just above the nucleus remains in place, and, through this, the spindle moves to the pole. The sperm enters through the exposed endoplasm of the antipolar field.

Two polar bodies are given off. The segmentation nucleus is formed and the first cleavage furrow appears passing through the pole (Fig. 26*d*). As the division proceeds an antipolar lobe appears. Later it is absorbed into one blastomere. It reappears twice more during the cleavage.

The first cleavage is into unequal parts. The second cleavage divides the smaller cell into equal parts or nearly so and divides the larger cell into unequal parts of which the smaller is slightly larger than the other two small cells. The third cleavage is dexiotropic and it is approximately equal, although the micromeres of the first quartet are slightly smaller than the macromeres. The later cleavages are like those of other annelids. A section through the 16-cell stage shows that the materials of the egg have been sorted out to the various cells.

The action of the centrifuge on the egg of Chaetopterus has been studied by Lillie ('09) and by Morgan ('10). If the eggs are centrifuged before the egg-nucleus breaks down the three characteristic strata appear. They are less sharply defined than when the egg is centrifuged after the nucleus is dissolved. The differences in the results may be owing to the more fluid condition

of the egg in the latter stage, following the dissolution of the nucleus.

When centrifuged after the polar spindle has formed, the eggs fall at random in the centrifuge, and the three characteristic strata may lie in any position with respect to the primary axis. When the centrifugal force is not very strong the nuclear material shrinks into a sort of ring (Fig. 221*c*), but when the centrifuging is stronger or more prolonged three well-defined layers are produced. The first cleavage passes through the pole and divides the egg into unequal parts as it does in the normal egg. The smaller cell may contain all the fat or most of the yolk and some of the clear zone, or parts of any of these. In other words, the cleavage plane is not influenced by the stratification. The antipolar lobe appears at the usual time, and may contain the material of any stratum. If the egg is centrifuged when the maturation spindle is present, the spindle is not, as a rule, removed from its normal position with respect to the pole.

One additional fact of importance is shown, namely: that the ectoplasm is little, or not at all affected by the rotation. It has the same distribution as it has in the normal egg. The form of the cleavage of the centrifuged egg may, in part, be explained as due to its retention of the normal condition of the ectoplasm, but there can be no doubt that the position of the spindle, and its surrounding substance, is also an important factor in determining the cleavage pattern. Normal embryos develop from the centrifuged egg.

It is not without interest to note that despite the variety of visible materials in the egg of Chaetopterus, a normal embryo may develop after the interior materials have been redistributed and arranged in zones. Again, although this egg has the typical spiral cleavage of other annelids, it may develop normally after centrifuging. It is evident that this result is due to the retention of the normal type of cleavage despite the displacement of its visible materials. The evidence furnishes a very convincing argument in favor of the view that these materials are not formative stuffs.

Centrifuging the Eggs of Crepidula

Conklin ('16, '17) has shown that when the large eggs of the marine gastropod Crepidula are centrifuged, they become stratified in the same way as do the smaller eggs of other molluscs.

The yolk (and a small amount of pigment), that is thrown to the centrifugal (outer) pole, occupies about three quarters

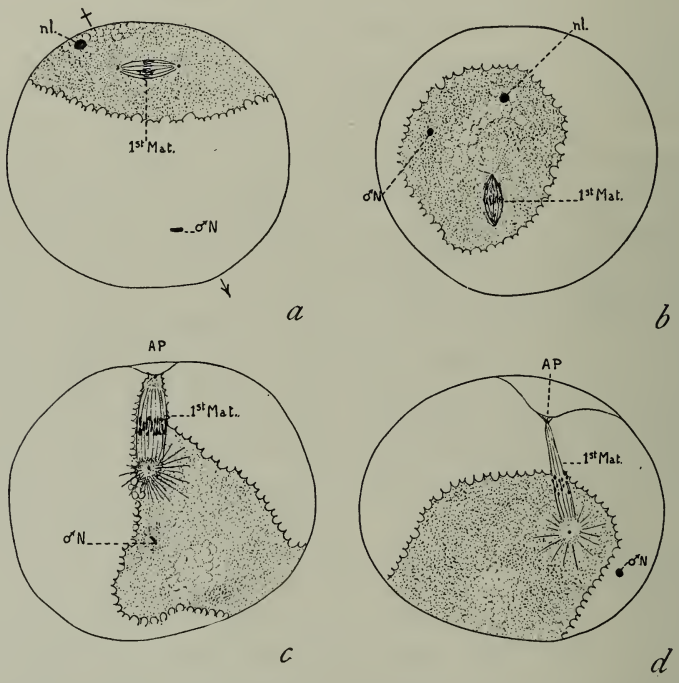


Fig. 222.—Centrifuged eggs of Crepidula. In *a* and *b*, the polar spindle has been moved from the pole. In *c* and *d*, it is still attached, but has been drawn out. (After Conklin.)

of the volume of the whole egg. The clear middle zone constitutes a little less than one quarter, and the fat (oil) zone only about one sixty-fourth of the volume of the egg. There is a tendency for the eggs on the centrifuge to rotate with the pole toward the center of rotation; but most eggs fall at random, and, retaining their first position, become stratified in all possible planes.

When the maturation spindle is just forming, it may be moved along with the more protoplasmic substance which surrounds it

(Fig. 222*a*), but after the metaphase stage is reached, the spindle appears to be so strongly attached to the pole of the egg that it is difficult to drive it from this position (Fig. 222*c*). The sperm-nucleus that has entered by this time, usually in the antipolar hemisphere, is not moved, and may be found in any one of the stratified layers. Conklin interprets this to mean that the sperm-nucleus has become so firmly imbedded or attached to the framework of the egg that it cannot be displaced.

When the earlier maturation spindle is present it may be carried from its polar location deeper into the egg, but it returns to the pole after removal of the eggs from the machine, and the polar body is found in the normal position. If the eggs are centrifuged at a still earlier stage, in the prophase stage of the spindle, and are kept rotating on the machine while the polar body is forming, the first polar spindle may come to the surface at some point other than at the pole, and there give off the polar body. This result is significant in showing that there is nothing in the "polarity" of the egg itself that determines the peculiar division that gives rise to the polar body, since the polar body may, apparently, be formed at any point of the surface to which the first maturation spindle is carried. The result also shows that there is no difference between the two poles of the spindle, since the normal inner pole may become the outer one.[1]

The second polar spindle in its earlier stages, may also be driven from the pole, but in its later stages, like the first spindle, it seems to be attached to the pole and cannot be moved. When the spindle is moved, it takes up a new position in the clear zone near the oil field (Fig. 223*a*). It will return, even from the antipolar region, to the pole and extrude the second polar body there unless the egg is kept on the centrifuge during the formation of the polar body. Under the latter conditions, the second polar body may be given off at any point on the surface of the egg even at the antipole. "Most polar bodies that are formed during

[1] The growth of the pronuclei appears to be influenced by the kind of material in which they lie. Normally the egg-pronucleus lies in the more protoplasmic region near the pole, and is larger than the male-pronucleus that is advancing to meet it in the yolk hemisphere. But when the egg is centrifuged, in the anaphase of the second polar spindle, the yolk may be driven into the polar region without dislodging the egg-pronucleus, which then remains smaller than the sperm-nucleus that is advancing through a region largely protoplasmic.

centrifuging, and lie at a distance from the animal pole, are larger than normal ones. They may vary greatly in size. The size of a polar body (or of any cleavage cell) depends upon the position of the mitotic figure at the time of cell-constriction, since the partition wall between daughter cells always goes through the equator of the spindle. When one pole of the spindle is pressed against the cell membrane, as in the maturation divisions, the

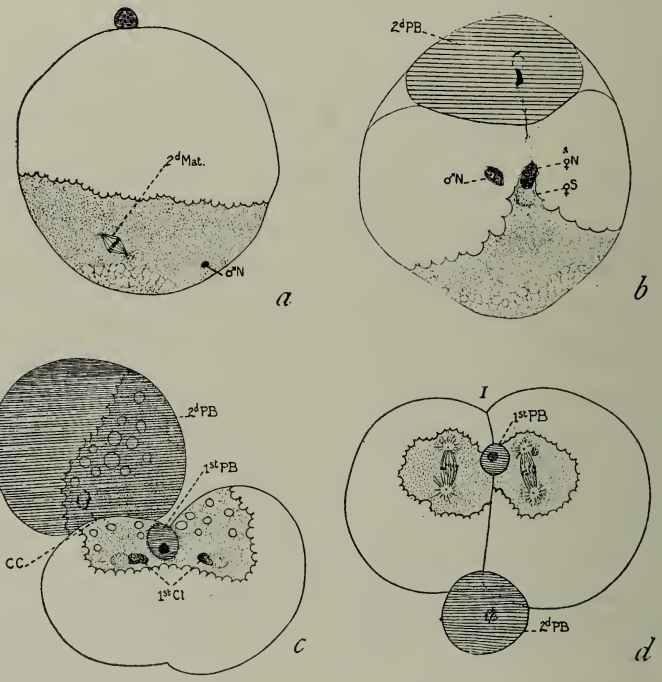


Fig. 223.—Centrifuged eggs of Crepidula. In *a*, the second polar spindle has been driven to the opposite pole of the egg. In *b* and *c*, the polar body is enormous, owing to the position of the spindle that had been driven from the pole. In *d*, the second large, polar body had formed at the antipole, but the segmentation-nucleus returned later to the polar region of the egg before the first cleavage took place. (After Conklin.)

size of the polar body depends upon the length of the spindle. The extremely small size of normal polar bodies is due to the fact that the maturation spindle continually grows shorter during the later stages of mitosis, and, whenever giant polar bodies are formed, it is due to the median position of the spindle in the cell or to its elongation if it is attached to one pole" (Conklin).

If the first polar spindle is stretched, the first polar body is

large; if the second polar spindle is stretched the second polar body is large. In the normal egg, the first polar body usually divides once and only once. But the giant polar bodies were never seen to divide, even although they may contain some or all of the visible constituents of the egg. They may be as large as or even larger than the rest of the egg (Fig. 224). Furthermore they do not divide even if given off at other regions than the pole of the egg. The old explanation, that the polar bodies do not divide because of their size, cannot be the only condition that accounts for their failure to divide.[2] Results to be described later

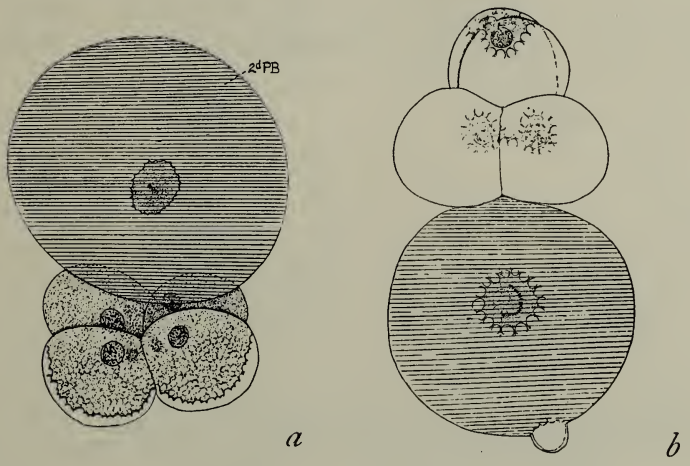


FIG. 224.—The second "polar body" in these two eggs is larger than the "egg" itself, which has, nevertheless, divided nearly normally. (After Conklin.)

show, Conklin thinks, that they do not develop because they do not contain a sperm and its aster.

In a polyclad worm, Prostheceraeus, Francotte ('98), had found that the first maturation division (polar body division) sometimes divides the egg into nearly equal cells. Each such cell may be entered by a sperm and develop. The egg gives off a second small polar body, undergoes cleavage, and develops as far as the gastrula stage. In this case the development of the polar body is clearly due to the entrance of the sperm. On the other hand in Crepidula the sperm enters the egg before the first polar body

[2] The first polar body does divide in *many cases,* even when extruded before fertilization.

is given off. The entrance of one sperm prevents the entrance of more sperm even into the large polar body. This "immunity" brought about by the entrance of the first sperm does not confer upon the egg or the polar body the power to develop, even although in Crepidula the sperm has been in the egg three hours before the first polar body is given off. A similar situation was observed by Ziegler ('98). He found that if an egg of Echinus was compressed by a cotton thread in such a way that the part into which the sperm had entered was connected by only a narrow bridge of protoplasm, the portion containing the egg-nucleus failed to divide, even although the nucleus underwent a series of division phases. The part containing the sperm, however, did divide. Evidently something brought in by the sperm, that does

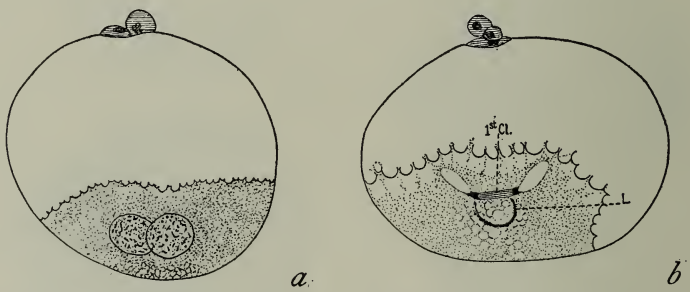


FIG. 225.—In *a*, the two pronuclei have been driven into the antipole. In *b*, the segmentation spindle has formed in the same position. Both eggs had been centrifuged after the extrusion of the second polar body. (After Conklin.)

not diffuse through the egg, brings about division. In Crepidula only that part of the egg that contains a sperm divides, and may be called the egg, while the other part (either polar or antipolar) that does not contain a sperm, is called the polar body, because it does not divide. The result shows, as Conklin points out, that one of the most essential factors of fertilization is the introduction of a division center into the egg by the spermatozoön, rather than a diffuse chemical action of the sperm.

One of the most puzzling results of Conklin's work is the return of the nucleus and its surrounding protoplasm to a position nearer the polar end of the egg. The subsequent divisions are normal, even in cases where the second polar body is larger than the egg itself. Under these circumstances the "polar region" does not mean the original pole of the egg, since this may be

separated off and lie in the polar body, but the polar region is now that part of the egg nearest to the original pole. For example, in Fig. 223*d* the second polar body was given off at or near the antipole, yet the first cleavage started at that part

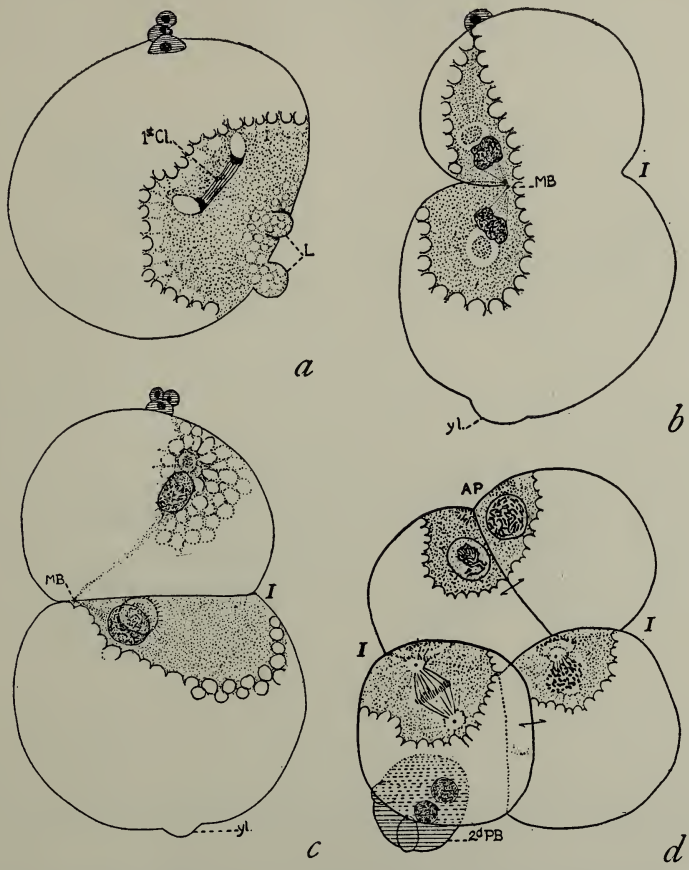


FIG. 226.—*a* and *b*, cleavage of eggs centrifuged after the extrusion of the two polar bodies; *c*, centrifuged during the two-cell stage, and *d*, during the four-cell stage. (After Conklin.)

of the cell nearest the original polar field. The spindles for the second division lie just below the true pole, as determined by the location of the first polar body.

In Fig. 223*c* the second polar body is as large as the egg—the mitotic figure lies near the polar end of the egg. In Fig. 224*a* the second polar body is much larger than the egg, which is divided

into the first four cells with the nuclei at the polar end of the cells. In Fig. 224*b*, the second polar body (showing the yolk-lobe) is at the antipole, while the nuclei of the egg, which is divided somewhat abnormally into four cells, lie near the polar end of the cells where the next division is expected to occur. These same relations are shown also by later cleavage stages which are normal in all essential respects.

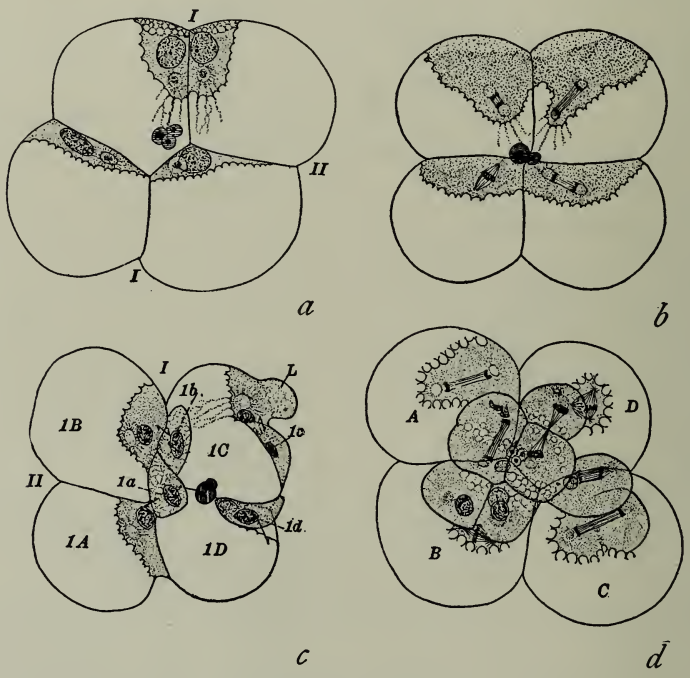


Fig. 227.—*a*, egg centrifuged in the four-cell stage; *b*, *c*, *d*, later cleavages of similar eggs in which the cleavage spindles and nuclei had returned to the original polar region before division. (After Conklin.)

The two pronuclei and also the segmentation spindle of the egg of Crepidula can be moved by centrifuging (Fig. 225*a*, *b*). If, as shown in Fig. 226*a*, *b*, the spindle has been carried into the egg so that it lies *vertically* to its normal position, the cleavage plane may be in an equatorial plane. After the division the nucleus and the centrosphere in the cell below the equator (antipolar blastomere) move to a position as near as possible to the polar end of the cell (Fig. 226*c*) and a similar though more

restricted movement takes place in the upper cell (polar blastomere). The next division is therefore meridional (Fig. 226*d*). Each of the four cells so formed gives rise to three sets of micromeres "precisely as in the normal egg, except that the micromeres in the cells below the equator are not able to reach the animal pole though they move as far as possible in that direction."

Eggs centrifuged in the 4-cell stage may have their nuclei displaced as shown in Fig. 227*a*, *b*. When the next cleavage takes place the nuclei tend to return to their normal position in the cells, and often accomplish this, so that the four micromeres are normally placed. At times, however, two of the micromeres may form at some distance from the pole as in Fig. 227*c*. This result shows again that the kind of division that takes place is determined by the conditions surrounding the mitotic spindle rather than by any predetermined region of the egg.

Finally, it is possible, by centrifuging the egg after the first quartet of micromeres is formed, to carry the nuclei in the macromeres to the antipole and hold them there during the following division by continuous centrifuging. The second quartet of micromeres then forms at the antipole (Fig. 227*d*). If released after this division the nuclei in the macromeres return to the polar ends of the cells and give off the third quartet around the first quartet of micromeres.

Centrifuging the Egg of Ascaris

The eggs of the nematode of the horse, Ascaris megalocephala, have been centrifuged by Boveri and Hogue ('09), Hogue ('10), and Boveri ('10). In order to bring these results into harmony with the earlier work on other eggs, two facts must be borne in mind: first, that what Boveri calls the "yolk" is driven to the centripetal pole, and the "brown granules" to the opposite, (outer pole); second, that after the first polar body has been extruded (and it is at this time that the egg is centrifuged) the membrane has been formed and the egg has shrunk, leaving a large space filled with fluid between its surface and the membrane. In this fluid the egg is free to move and when centrifuged, it orients in many cases to the driving force, so that its polar axis coincides with the axis of rotation. Its antipole (where the "yolk" accumulates) lies, as stated above, toward the axis of rotation.

Greenish "yolk" (fat?) granules are present in the Ascaris egg, sometimes distributed more or less evenly throughout, sometimes accumulated in one-half. When the ripe eggs are rotated slowly for a short time, and then examined in the tube, it is found that the greenish substance is at that end that lies toward the center of rotation. The nature of this yolk, so-called, appears, at least from its behavior on the centrifuge, to be more like the oil or fat of other eggs.

When the unripe eggs are centrifuged, the greenish colored yolk is driven into one hemisphere which it completely fills, the other hemisphere remains clear. Large clear "vacuoles" lie between. These unfertilized and centrifuged eggs may remain alive in this condition many weeks without the constituents redistributing themselves.

When ripe eggs are centrifuged at 3800 revolutions per

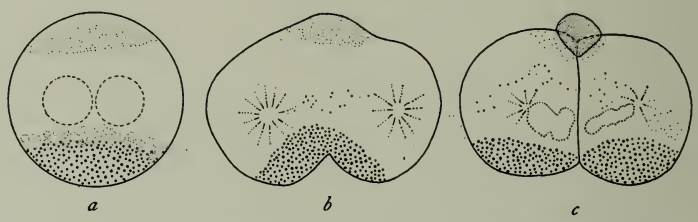


Fig. 228.—Centrifuged egg of Ascaris, and first cleavage of same. (After Hogue.)

minute, the stratification is accomplished in half an hour. The lightest constituent, the yolk, forms a cap (Fig. 228*a*) nearest the axis of rotation. Next to the yolk is a layer of large spheres. The middle zone is filled with clear protoplasm. The fourth zone is formed of fine, brown granules. The two pronuclei lie in the protoplasmic zone; the spindle develops later in this zone. When the egg is being strongly centrifuged, it becomes flattened in the direction of the force. When the spindle is present, or forms in the egg during centrifuging, its position is not affected unless a high speed is applied for a long period. Consequently, when the division takes place it bears no constant relation to the stratification except in those eggs whose axes coincide with that of the centrifugal force. But if the eggs are centrifuged 3800 revolutions per minute, and not removed from the machine until 10 or 15 minutes before cleavage sets in, and then placed at once

in a warm chamber (30 degrees C.), the egg divides at right angles to the stratification.[3]

If eggs are not put into the machine until the first spindle is formed the position of the spindle is not changed, and the division bears no relation to the stratification.

If eggs with two pronuclei are centrifuged and kept revolving (3800 revolutions per minute) until the cleavage is finished, then,

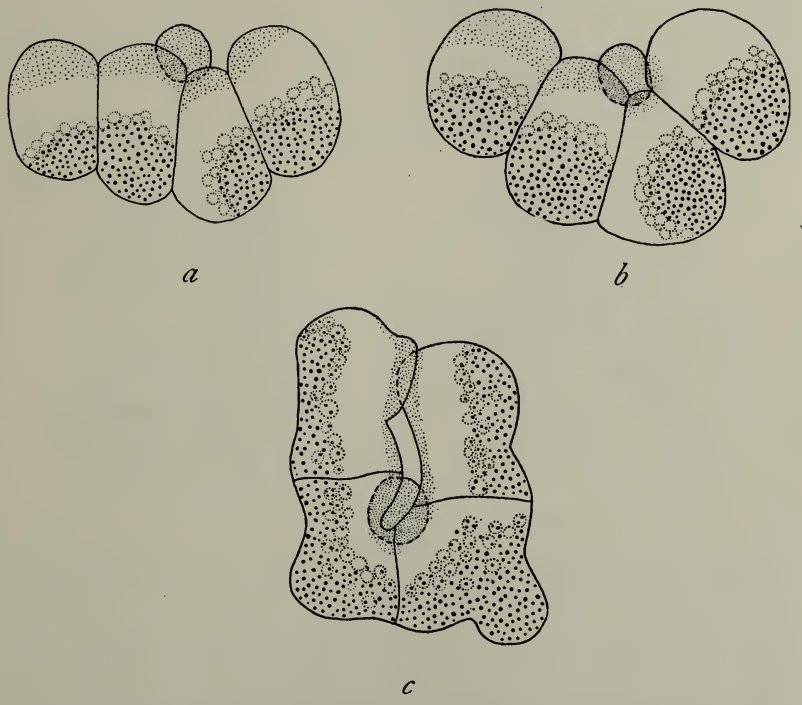


Fig. 229.—Four-cell stage of centrifuged egg of Ascaris, showing, in *c*, rearrangement of blastomeres as in normal egg. (After Hogue.)

as stated above, all the eggs divide at right angles to the stratification. In this case the position of the spindle must be affected and Hogue thinks this is due to the flattening of the egg rather than to an effect of the centrifuge. The result is like that produced

[3] If, however, they are not put at once into the warm-chamber but kept at room temperature, the eggs may divide in any plane with regard to the stratification. This must mean that the spindle returns to a "preferred" position, i.e., in the original axis of the egg.

on the centrifuged frog's egg (Morgan), where the flattening persists for a longer time. Even if the position of the spindle has been changed in the Ascaris egg on the machine, it returns to its normal position in the direction of the polar axis as soon as the egg becomes spherical after removal from the machine and allowed sufficient time by keeping it at room temperature. The spindle may then be in any position as regards the stratification, but the division of the egg is always at right angles to the slide. This results from the flattening of the egg coat due to drying. Normal embryos may develop irrespective of the distribution of the centrifuged materials.

When the egg divides at right angles to the stratification

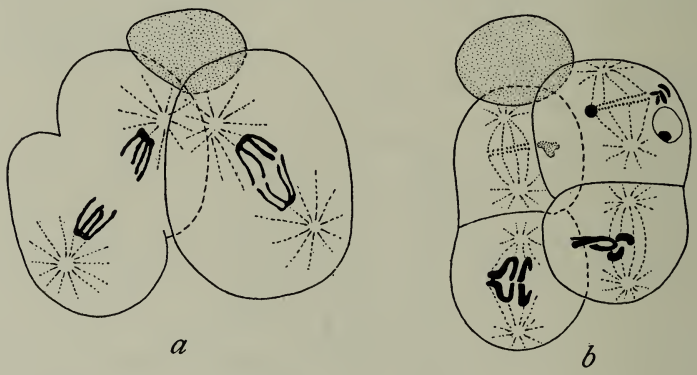


FIG. 230.—Two- and four-cell stages of centrifuged egg of Ascaris, showing in *b*, the diminution of the chromosomes in the two upper of the four cells. (After Hogue.)

(Fig. 228*b*), the plane of cleavage separates two like halves, but the division does not always pass through the green cap (Fig. 228*c*) which is cut off from the egg as a "ball." This ball does not divide further and is left outside the developing embryo. The rest of the egg continues its divisions, but these eggs rarely form normal embryos. The second division is nearly parallel to the first (Fig. 229*a*) and is, therefore, unlike the second division of the normal egg. The four blastomeres may stand in line, or more often in an arc. Later they shift their position (Fig. 229*c* and 230*a*, *b*) until the two outer-lying cells come in contact. This meeting is sometimes on the side next to the ball (Fig. 229*c*), sometimes in the opposite direction. After the next division into eight, the blastomeres may lie in two parallel rows (Fig. 231*a*) if

the egg shell is drawn out; or lie more irregularly (Fig. 231*b*) if the egg is spherical.

At the division of the four cells into eight a remarkable difference is apparent in the behavior of the chromatin on the spindle of two of the cells (Fig. 230*b*). In these two cells—those next to the ball—there is a "chromatin diminution," while none takes place in the other two cells.

In the normal egg one of the first two blastomeres is "somatic," the other gives rise to the germ-cells as well as to some somatic cells. In the centrifuged eggs, on the contrary, the descendants of both the first two blastomeres give rise to germ-cells; in other words, there are two germ-tracks instead of one. This difference

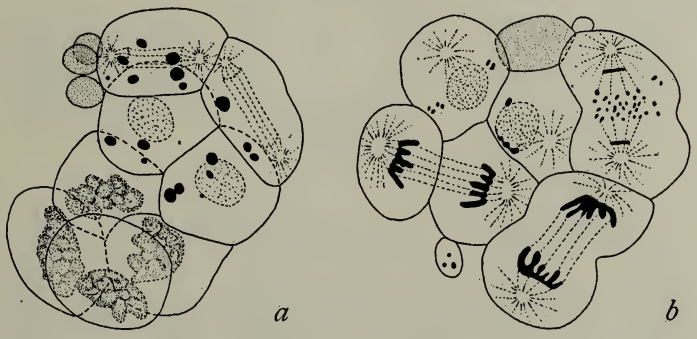


FIG. 231.—Eight-cell stages of eggs that had been centrifuged before cleavage. (After Hogue.)

becomes manifest only in the transition from the 4- to the 8-cell stage. In the centrifuged eggs up to the 4-cell stage (Fig. 230*a*) there is no chromosome diminution. At the next stage (Fig. 230*b*), as stated above, diminution occurs in two cells, while in the normal egg it occurs in three cells at this time (in A and B and EMST, Fig. 14).

Boveri has pointed out certain relations between the cleavage of those eggs that divide at right angles to the stratification (eggs that are also oriented) and the normal division of the Ascaris egg that makes clear this difference in the number of cells showing chromatin diminution. If the normal egg is watched at the time when the first segmentation spindle is forming, it is observed to elongate at right angles to the polar axis, but as the spindle develops it rotates (as in Ascaris nigrovenosum) so that before

division it stands in the polar axis. The first division of the Ascaris egg is therefore across the axis, and corresponds, in a way, to the third division of most other eggs. The antipolar blastomere gives rise to the germ-track cells. But when the egg is kept on the centrifuge until it is about to divide or until it has divided, and if it lies with its polar axis in the direction of the centrifugal force, the first division will be at right angles to that of the normal egg (Fig. 232*b*). Consequently the protoplasmic material that goes into the antipolar cell of the normal division is here divided between the first two cells, and if this is the material that determines diminution of the chromosomes, then it is due to take place in the descendants of the two antipolar cells of the centrifuged egg. This is in accord with the discovery that Boveri and Stevens had previously made, showing, from an analysis of the

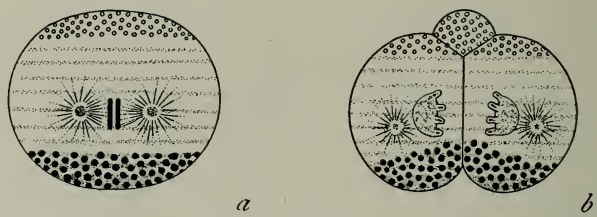


FIG. 232.—*a*, centrifuged egg that was kept on the machine until the first division (*b*) had taken place. (After Boveri.)

behavior of dispermic eggs, that it is the protoplasm of the cell that determines where diminution will occur, and not any peculiarity of the chromosomes, or the time of their division.

CENTRIFUGING THE EGGS OF THE FROG

The eggs of the frog were first centrifuged to determine whether the action of gravity is necessary for development. Later the injurious effect resulting from prolonged centrifuging of the egg during its development was studied. Later still a higher rate was used, for a shorter time, in order to bring about a new distribution of the materials of the egg. It is the latter results mainly that concern us at present.

Morgan ('06) centrifuged eggs of Rana sylvatica and Bufo lentiginosus, at the rate of 1600 revolutions per minute. The eggs occupied the outer ⅔ of the tube at a distance of 10 to 12 cen-

timeters from the center of the rotation. The best results with the frog's eggs were obtained after 7 minutes' rotation, and with the toad's eggs after 3 minutes. A slower rate than this produced no visible effect on the eggs, while a longer period of rotation produced changes that prevented normal development. The eggs had been fertilized before rotation began. They oriented on the machine with the black pole toward the center. The eggs became somewhat flattened in the polar axis, as a result no doubt of the pressure of the mass of eggs on those farthest from the center of rotation. The eggs may retain their flattened shape even after

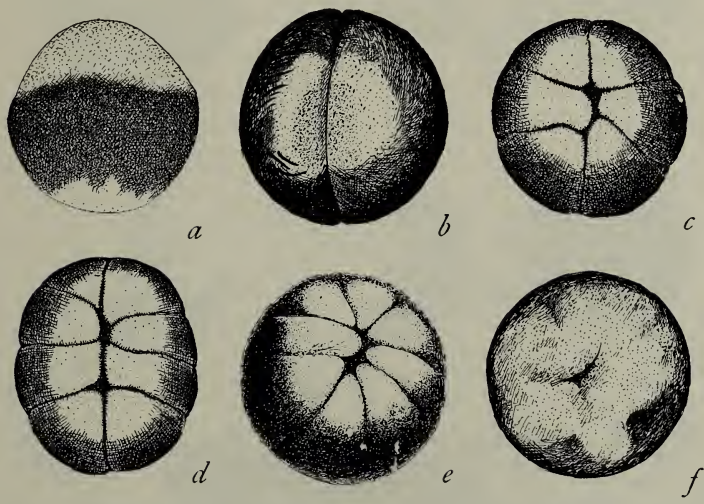


FIG. 233.—*a*, centrifuged egg of the toad; *b–e*, later cleavage of same, as seen from the pole; *f*, late blastula as seen from the pole. (After Morgan.)

removal from the tubes. The black hemisphere becomes lighter in color and finally quite white. The black pigment granules of this hemisphere are driven into the interior of the egg, forming a layer some distance below the upper pole (Fig. 235*a*, *b*), and just above the yolk. The yolk granules of the upper hemisphere are also driven into the denser yolk of the white hemisphere. Sections of these eggs show a clear protoplasm in the polar field (Fig. 235*a*).

The first cleavage is through the pole and divides the egg as a rule into two equal parts—but sometimes unequally (Fig. 233*b*). The second cleavage is at right angles to the first and also through

the pole. The third divisions are also "vertical" dividing the egg into eight equal or subequal cells (Fig. 233*d*, *e*). The fourth cleavage may also be vertical, but is more often "horizontal," cutting off eight small polar cells. As the cleavage continues, the white polar region expands over a larger area (Fig. 234*a*). The dorsal lip of the blastopore appears below the equator in

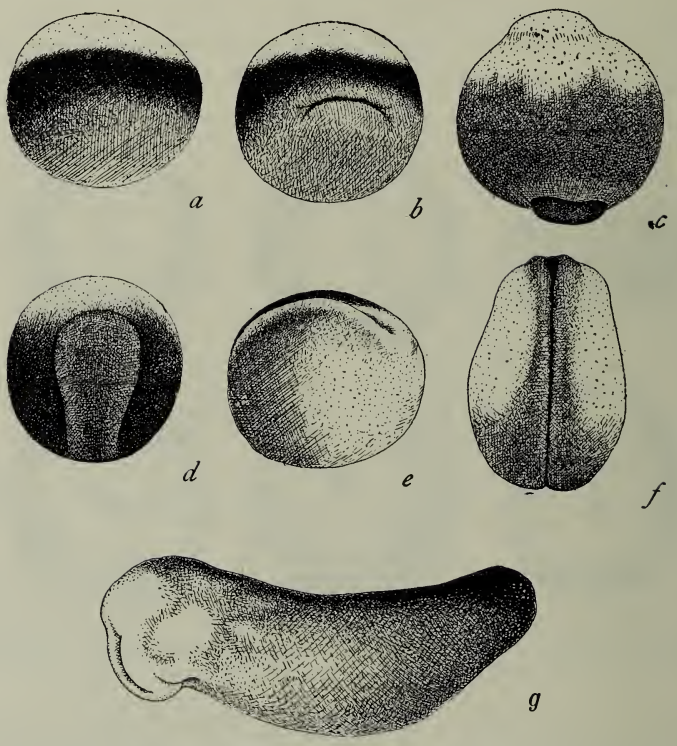


Fig. 234.—Later stages of centrifuged egg of toad. (After Morgan.)

the black zone and advances over the "yolk-hemisphere" as in the normal egg (Fig. 234*b*, *c*).

When the neural plate appears it extends as far forward as the edge of the white field, i.e., just above the equator of the egg (Fig. 234*d*). As the neural fold closes in, the white polar field becomes drawn backward and upward on the sides of the embryo (Fig. 234*e*). The original pole of the egg lies beyond the anterior end of the young "embryo." The darker equatorial ring of

pigment becomes incorporated in the nervous system, in the dorsal surface and in the posterior end of the embryo.

The normal tadpoles from the centrifuged eggs have been kept for several weeks or longer (Fig. 234*g*). The white ventral and lateral regions gradually become darker as new pigment develops. The result shows that the redistribution of the materials, carried as far as described above, does not interfere with normal development.

Our knowledge of the chemical composition of the stratified

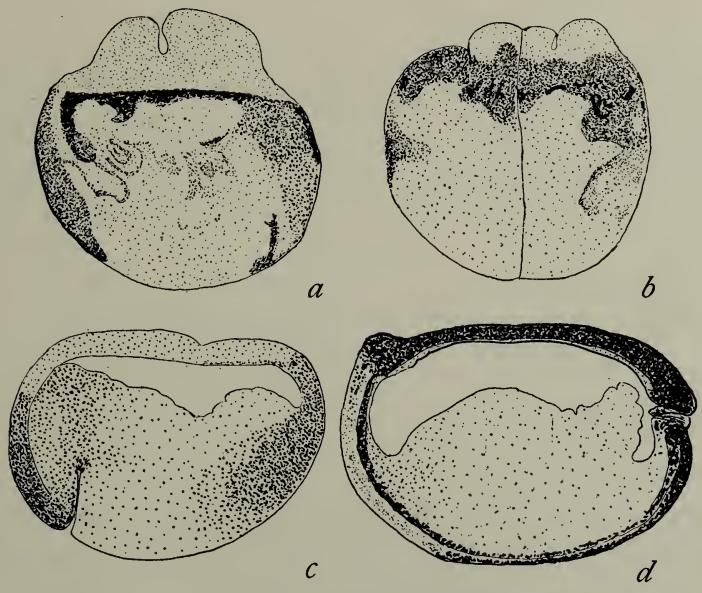


FIG. 235.—*a*, section of centrifuged and unsegmented egg of toad; *b*, section of two-cell stage; *c*, section of gastrula; *d*, section of young embryo; the top of the egg corresponds to the antero-ventral region of the embryo. (After Morgan.)

substances of the centrifuged egg of the frog rests largely on results obtained by McClendon ('09, '10) and Jenkinson ('15). Ripe ovarian eggs of the frog were squeezed through bolting cloth, the material was quickly weighed and centrifuged. The materials separated into three layers that were identical in appearance and relative volume, with those seen in the centrifuged living eggs. The three layers were separated and examined chemically. Each was dried over sulphuric acid in vacuo, and the water content calculated. The dry material was extracted first with ether and

then with boiling water. The final residue was examined for phosphorus content. In the following table the per cent of water is given for the three layers.

TABLE XXIV

Layer	Water	Solids
Fatty.....	50	50
Middle.....	82	18
Heavy.....	48	52

The middle layer contains the most water; the heavy layer the least. The consistency of the three layers is largely determined by their water content; the middle layer being more fluid; the fatty layer more like butter, and the heavy layer more like cheese. In the next table the per cent of the extracts and residues is given.

TABLE XXV

Layer	Ether Extracted	Alcohol Extracted	Water Extracted	Final Residue
Fatty.....	80	4	8	8
Middle.....	7.5	11.5	60	21
Heavy.....	24	6	10	60

All the layers were extracted by the ether and alcohol; the fatty layer contained most, the middle layer least. The highest per cent of salts is present in the middle layer, the lowest in the fatty layer. The highest per cent of protein is contained in the heavy layer, the lowest in the fatty layer.

The analysis of the phosphorus distribution furnishes an index to the principal constituents. There was not enough to weigh in the ether extract. This extract was "chiefly fats with a trace of lecithin and probably some cholesterin. Lecithin is relatively deficient in the fatty and middle layers. The final residue of the heavy layer is probably pure vitellin." The yolk of the frog's egg is a phosphorized protein, probably a vitellin that is bound up in some way with lecithin.

The structure of the frog's egg with respect to the substances stratified by the centrifuge, has been studied by Gurwitsch ('04,

'09), Morgan ('02, '06), Konopacka ('08), McClendon ('10) and by Jenkinson ('15). The following description is that given by McClendon, because he has made use of a greater variety of methods in studying the egg. If an ovarian egg is burst and its contents smeared on a slide and studied at once, large oval or round granules, smaller, round, fat droplets and minute pigment bodies are found imbedded in the protoplasm. If such a smear is fixed in osmic acid the fat droplets stain black. They can also be stained by Soudan III, both reactions indicating their fatty nature. In such preparations the droplets can be seen to run together to form larger fat drops. It is these larger spheres of fat that collect at the fat pole after centrifuging. The pigment-granules have been shown by McClendon to be melanin; the yolk-granules are made up of lecithin (6 per cent) and batrachiolin, a nucleo-albumen (94 per cent); and the fat droplets are composed of a mixture of liquid fat, a solid fat and a yellow lipochrome.

The granules of different kinds are normally surrounded by, or suspended in, the protoplasm. Whether this protoplasm is itself alveolar or reticular is a mooted point that need not be discussed here. After centrifuging, the middle layer which contains a larger part of the protoplasm has a reticular structure that is supposed both by Gurwitsch and by McClendon to be an artifact. Possibly to some extent the holes in the reticulum may be spaces from which the yolk, pigment and fat were driven out, now filled with a more liquid substance. This view is, however, extremely hypothetical.

The injurious effects of prolonged centrifuging have been noted by several observers. It is especially noticeable in large eggs like those of the frog. Some of the injury probably results from a disturbance of the mitotic figure. The spindle may be distorted or carried to part of the egg where normal distribution of the chromosomes is interfered with. The yolk may be so compacted in the lower hemisphere that the cleavage plane cannot pass through it, or the mitotic figure may not be able to penetrate into it, hence the frequent formation of spina bifida. It has been found (Konopacka, Jenkinson), that at certain stages of development, the injury caused by centrifuging is greater than at other stages. It is difficult to attribute these differences to any one effect in particular, but they may problematically be referred to one or

another of the effects just mentioned. The substances of the mitotic figure and its immediate envelope appear denser or more solid than the protoplasm in general. Hence this material moves as a whole under the influence of the centrifuge. Neither the yolk nor the pigment can be driven through it, consequently not infrequently there may be found a small accumulation of yolk and pigment on the centripetal side of the mitotic figure, and yolk may even accumulate in the funnel-like spaces between those fibres of the aster that open out in a centripetal direction.

Compression of the mitotic figure is sometimes seen in large eggs like those of the frog (McClendon). This may safely be attributed to the compression in the direction of the centrifugal force of the more solid material of the figure. The resulting figure gives the impression that the rays are real, if only temporary, structures in the cell.

The chromosomes cannot be separated from the rest of the mitotic figure. They appear to be firmly imbedded in it or even, as some writers think, attached to the denser central spindle-fibres of the figure.

Konopacka ('08), has studied the effect of centrifuging various early stages and has described the resulting abnormalities. Jenkinson ('15) also has described some of the common abnormalities produced by centrifuging. He also examined the chemical properties of the material of the three zones obtained by crushing the eggs and centrifuging the materials at the rate of 3200 revolutions per minute for twenty minutes. He finds that the centripetal layers contain fatty substance, protein, and a little glycogen. Part of the fatty substance is lecithin. The second layer contains a good deal of glycogen, and a small quantity of fatty substance (part of which may be lecithin), and solid protein. The third layer contains pigment, yolk, and some fat, but no glycogen. The relative volumes of the first and second to the third layer of "egg-pulp" is as one to four. Oedquist ('22) rotated frog's eggs at 2500 revolutions per minute and found evidence that changes in viscosity take place after fertilization. It is noticable one hour after fertilization, and increases till shortly before the first cleavage appears, etc.

Centrifuging the Eggs of the Leech, Clepsine

In the eggs of the leech, Clepsine sexoculata, there appears at the poles, after the polar bodies have been given off, a ring of material called the polar ring, and also another disc of material

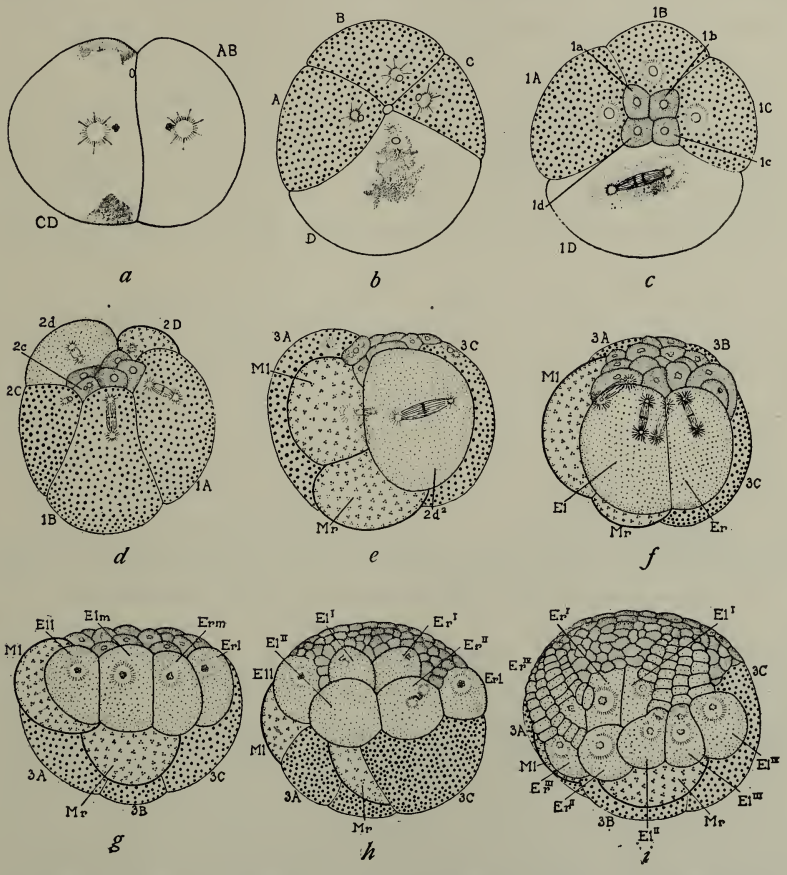


Fig. 236.—Normal cleavage of the egg of the leech, Clepsine. (After Schleip.)

at the antipole. These materials from the polar fields subsequently pass, for the most part, into the large D-quadrant.

The formation of the polar ring and the cleavage of the egg have been described by Whitman ('78), Vejdovský ('88), Schleip ('14). When the first polar body is about to be given off (after the entrance of the sperm) the egg is elongated in the direction

of its primary axis (Fig. 237*a*). After the second polar body has been given off, and while the two pronuclei are coming together in the segmentation spindle, the polar and antipolar fields appear (Fig. 237*b*). Just before the first cleavage furrow is about to appear, the polar ring and the antipolar disc sink deeper into the interior (Fig. 237*c*), and the cleavage furrow cuts the egg in such a way that practically all of the materials from these two sources come to lie in the larger (CD) cell (Fig. 236*a*).

The cleavage follows the spiral type, characteristic of most annelids, with some deviations (Fig. 236, see also Fig. 144 giving the cell lineage of Tubifex). The first division is unequal, the cleavage plane passing to one side of the polar material which in consequence passes, as stated above, almost entirely into the

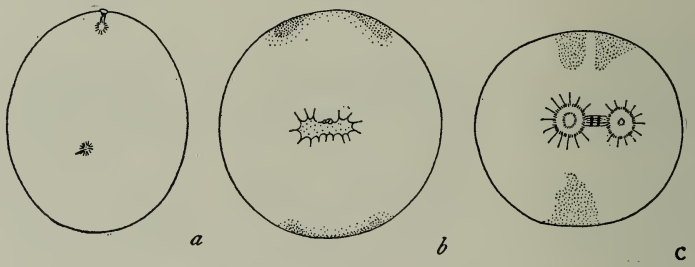


FIG. 237.—Sections of normal egg of Clepsine. In *a*, the first polar body is present. In *b*, the two polar fields have developed. In *c*, the segmentation spindle is present and the polar fields have begun to sink into the egg. (After Schleip.)

large CD cell. The antipolar material is also present in this cell, which is larger tha n the AB cell (Fig. 236*a*). At the next division the AB cell divides into nearly equal parts, but the CD cell divides unequally (Fig. 236*b*). There results one large D-cell containing most, but not quite all, of the polar and antipolar materials, and three smaller cells of unequal sizes. At the three successive divisions that follow (Fig. 236*c-f*), four quartets of micromeres are produced, but the spiral form of the division is less evident than in the eggs of other annelids. The second micromere, 2d, is much larger than its sister micromeres of the D-quadrant (Fig. 236*d*).

Before the polar bodies have been given off, the yolk granules are evenly distributed through the protoplasm. There is a thin layer of ectoplasm present. Fat-droplets lie amongst the yolk-granules. Schleip has shown that when an egg that has just

given off its polar bodies, but has not formed its polar fields, is centrifuged for ¼ to ½ hour, there appears a fat layer over the centripetal end (Fig. 238*a*). Beneath this cap there is a clear layer. The rest of the egg is filled with the yolk.

If an egg is centrifuged when the polar fields are present, the same three substances are stratified in the same way (Fig. 238*b*). In addition two "plasma masses" are seen (in sections) that are

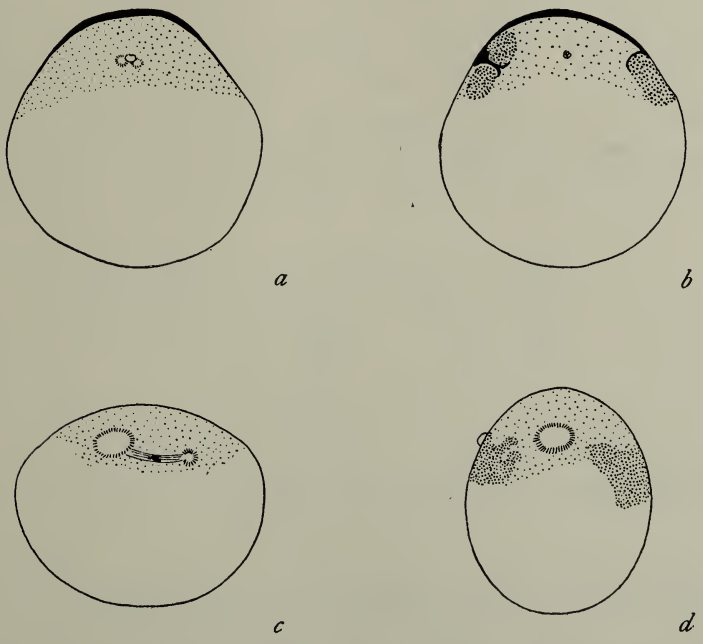


FIG. 238.—Centrifuged eggs of Clepsine. *a* and *c*, sections through eggs at right angles to polar materials; *b* and *d*, sections through eggs cut through the polar fields. (After Schleip.)

the polar and anti-polar materials which have for the most part remained *in situ*. One of these still retains its characteristic ring-form. They lie in the clear zone near the yolk and at the surface.

Before the polar bodies are formed, the pole of the egg is its lightest end, since the egg usually turns with this end uppermost. During the time of extrusion of the polar bodies the egg does not orient in this way, consequently it might be expected to fall in any position on the centrifuge.

After the polar ring has formed, the polar half is again lighter

than the antipolar half, since the egg, set free in water, orients with the pole upward. The egg has become spherical at this time. But Schleip states that the eggs do not turn in the machine with the pole turned towards the center, and he thinks this is due to the presence of two lighter substances, one at each pole. It is not clear, however, how this should make a difference in orienting in the machine and not when the egg orients in water. Nevertheless, it is evident from the position of the polar and antipolar fields in the centrifuged egg that the egg does come to lie in the machine at right angles to its primary axis (see *b* in Fig. 238). In other words, one side is turned in the centripetal direction, and the yolk is driven to the opposite side. Schleip attempts to explain the orientation as follows: The outer layer of the two pole-plasms sticks to the surface of the egg, and the ectoplasm covering (with

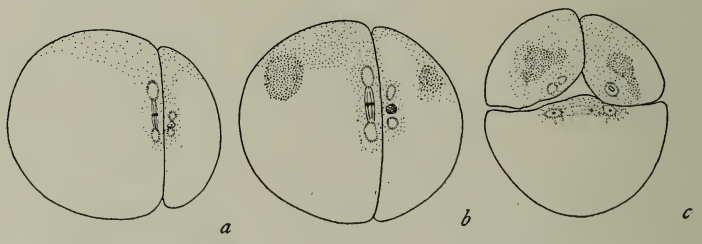


Fig. 239.—Two-cell stages, *a* and *b*, and four-cell stage *c*, of centrifuged eggs of Clepsine. (After Schleip.)

its adhering pole-plasms) places itself in equilibrium with the centrifugal force, i.e., it turns so that the line adjoining the two pole-plasms is at right angles to the centrifugal force (Fig. 238*b*). The evidence for this interpretation Schleip finds in the frequent position of the polar body next to the polar ring.

If an egg in the 2-cell or later stage is centrifuged, the pole turns centripetally and the primary egg-axis coincides with the axis of orientation, and the contents is stratified accordingly. The two polar fields have by this time united in the interior of the egg.

In eggs that are centrifuged before the polar fields formed, the first cleavage spindle lies parallel to the stratification. In an egg centrifuged after the polar fields have formed, the first spindle lies in the centripetal hemisphere between the polar and antipolar fields at about equally distant from each. The spindle lies

parallel to the stratification, hence at right angles to its usual position (Fig. 238*c* and *d*). At the second division (Fig. 239*a*) the two spindles are equally distant from the two polar fields and at right angles to their line of union, but at right angles also to the stratification. This means that they lie at right angles to the position of the first spindle. Exceptions exist, however.

At the third division of the normal egg, the first quartet of micromeres is formed, but there is no regular formation of micromeres in the centrifuged eggs. If formed at all, two only appear from the two cells containing the polar ring, but the other two cells divide irregularly.

Schleip concludes from this evidence that there is no indication of an ultimate organization of the egg that determines its form of cleavage independent of the distribution of the other substances of the egg, but rather that the cleavage is determined by the arrangement of the visible substances of the egg.

It must be admitted, however, that the evidence is scarcely sufficient to prove the point, since it is well known that other factors than an invisible structure of the egg, if such exists, affect the position of the spindles. The fact that two micromeres form only in the region of the polar field, does not suffice to show that their formation there is due to the pole-plasm, because in the normal egg this material has left the pole when cleavage begins, and can not be held responsible for the formation of the micromeres at the pole.

The cleavage of the eggs centrifuged in the 2- and 4-cell stages offers nothing of special interest. None of the centrifuged eggs produced embryos.

Centrifuging the Eggs of Tubifex

The eggs of the fresh water annelid Tubifex rivulorum have been centrifuged by Parseval ('22). He describes four zones: a fat cap, a granular protoplasmic narrow zone, a wide yolk zone, and a clear zone (Fig. 240). The presence of this clear, centrifugal zone beyond the yolk is something peculiar to this case, and not recorded in other eggs. Moreover, in some of his pictures more yolk and another clear zone is represented beneath the yolk. One can only conclude that the yolk in Tubifex has a different relative weight than that in all other eggs, or else, that the clear zone is an artifact, due, perhaps, to injury to the anti-

polar region. Something of this sort I have seen in the centrifuged eggs of Ilyanassa, where the egg may break, and set free much of the yolk, leaving a broken mass behind. The eggs of Tubifex orient on the machine (they are centrifuged collectively in the egg capsule) with the pole towards the center. The cleavage is influenced by the stratification, and is irregular for the most part, and only those eggs that have been rotated in the 2- or 4-cell stages occasionally give rise to normal embryos. Parseval

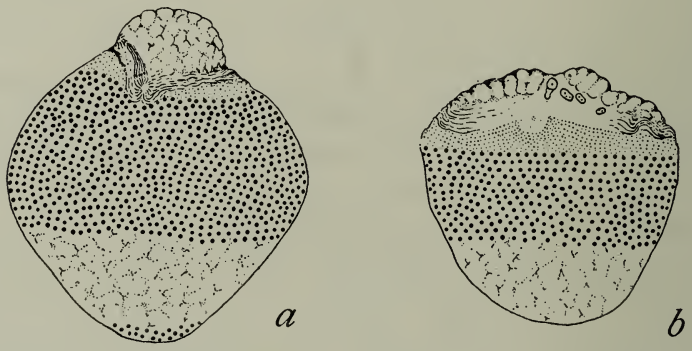


Fig. 240.—Centrifuged eggs of the annelid, Tubifex. (After Parseval.)

('22) attempts to correlate this result with the presence of the complete protoplasmic substance in the D-cell, which is unaffected by the first-formed, irregular division resulting from the stratification.

Additional Results Obtained by Centrifuging

The question has been raised as to whether the embryos that develop from centrifuged eggs are entirely normal in the sense that they would give rise to normal adults. The question has been answered in two cases. Parthenogenetic eggs of Hydatina senta have been centrifuged (Whitney '09, and Morgan '10). They continue to develop normally and produce normal adults (Morgan), both males and parthenogenetic females. Spooner also obtained normal embryos from the centrifuged eggs of a copepod (Fig. 241). These embryos became adult and laid eggs, which, needless to say, were normal in the distribution of their visible contents.

After removing the membranes from sea-urchin eggs (by the

well-known method of shaking violently in a small tube immediately after fertilization) Payne ('09) centrifuged the eggs. He then selected an egg in which the first division had been equatorial and separated the two cells. One of them contained the fat cap and protoplasm alone. This isolated cell formed a normal light-colored embryo of half size. The result showed that the yolk and pigment are not essential to the development of a normal pluteus. The other cell also developed in some cases, but less normally, the failure being due most probably to excess of yolk. Gatenby ('22) centrifuged the eggs of the annelid Saccocirrus, three to five thousand revolutions per minute for twenty minutes. The eggs showed the typical three layers. He reports that there

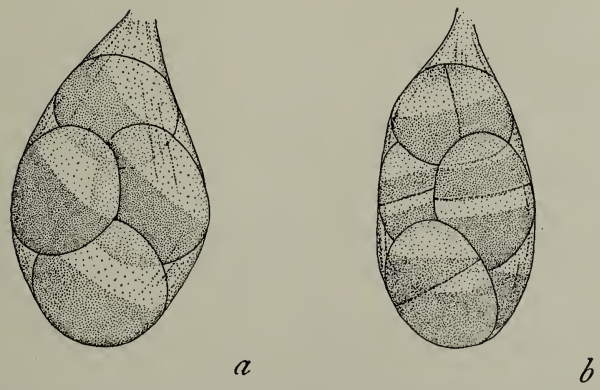


FIG. 241.—Centrifuged eggs of copepod, in egg-capsule. (After Spooner.)

is a fourth layer also, lying between the clear middle zone and the yolk that is composed largely of mitochondria.

Miss Beckwith ('14) centrifuged the eggs of a hydroid, Hydractinia and obtained normal, free-swimming blastulae.

The fertilized eggs of Styela have been centrifuged by Conklin ('24). They turn in their membranes, and the "vegetative pole" (the true pole of the egg) becomes placed centripetally, and the "animal pole" (the antipole) is centrifugal. At this time the yellow crescent or ring is already at or near the vegetative (antipole) end of the egg; hence centrifuging only accentuates the normal arrangement, and gives no answer to the question whether the pigment is organ-forming. But, "If the eggs are compressed by mutual pressure, or within capillary tubes, so that they cannot

rotate, the three substances (yellow pigment, clear plasma and gray yolk) may be displaced from their normal positions, and if they are so held until after cleavage begins these substances are abnormally distributed to the cleavage cells. Under these circumstances development is always abnormal." Conklin finds that the organs of the embryo from these eggs can still be identified by "their colors, shapes, sizes and histological characters" even when removed from their normal position. In eggs in which these substances have been dislocated by centrifuging, the larval parts, to which they typically give rise, are also dislocated. The larvae may even be turned inside out (as in the lithium embryos of the frog, etc.).

How far these results are to be interpreted as evidence of the organ-forming function of the centrifuged layers, and how far due to irregularities in the cleavage under pressure is not convincingly shown by this evidence. Until a detailed comparison can be made between these centrifuged and compressed eggs, and those that have cleaved under pressure alone, it is uncertain how far the abnormalities are due to one or to the other condition. It has been shown that the least alteration in the cleavage leads to the development of abnormal embryos, but whether, as stated above, these embryos differ in any important aspect from those centrifuged has not been stated. Whether the yellow pigment can be separated by centrifuging from the cytoplasm in which it lies, and may then determine the local differentiation of a region, must first be demonstrated in order that crucial evidence of their differentiating function may be established.

The eggs of Ciona have been centrifuged by Duesberg ('26), who finds that the failure of the centrifuged eggs to develop normally may be accounted for by the inequalities in the mitotic figures in some cases, or to the failure of the pronuclei to conjugate in others. The results depend on the stage at which the egg is centrifuged. The egg of Ciona is described as consisting of a "fundamental protoplasm," vitelline spheres (yolk), and mitochondria. When eggs are centrifuged during the formation of the polar bodies, the latter are given off, but a triaster forms in the egg. When centrifuged during the migration of the pronuclei towards each other, the union of the male and female pronuclei is often interfered with and the subsequent development is abnormal. A diaster (mitotic spindle) develops near each pro-

nucleus. When centrifuged during the formation of the segmentation spindle (45–71 minutes after insemination), numerous mitotic figures of which some are triasters develop in these eggs before they divide.

In all these classes of eggs there are some that do not segment, and show evidence of irregular mitoses. The segmentation of the triaster eggs, centrifuged during the polar body period, is into three parts of variable sizes. The segmentation of the egg centrifuged during the migration of the pronuclei is as a rule into two parts, each part with two nuclei, one a daughter nucleus of the female pronucleus, the other of the male pronucleus. The plane of division is variable, as is their subsequent development.

The segmentation of the eggs centrifuged when the segmentation spindle is present is extremely irregular, the egg dividing into several blastomeres. Duesberg thinks that the failure of the centrifuged eggs to develop normally is due to the irregularities in the mitoses and that the results do not show whether or not the formed substances of the egg are determinative.

In only one case have male germ-cells (spermatogonia) been centrifuged. Browne ('14) removed the testes from the bug, Notonecta, and centrifuged them. The testes were then preserved and sectioned. The cells showed the same stratification exhibited by centrifuged eggs.

The centrifuge has been used by Hegner ('09) to determine whether the so-called germinal protoplasm, that lies at the posterior end of the egg of many insects, is essential for the formation of the germ-cells that develop from this region, or whether the germ-cells will still develop if this material is thrown into other parts of the eggs. The eggs of a chrysomelid beetle were put into centrifuge tubes with the posterior pole turned toward the center. It was found that the so-called germinal protoplasm that lies at the posterior end of the egg was driven from its normal position into the interior of the egg. The embryos from such eggs were abnormal in many respects. The germ-cells appeared to be lacking. The result was interpreted as showing that, unless the germinal material is present at the posterior end to surround the nuclei that wander into this region, germ-cells are not produced. If this conclusion could be established without qualification, it would show that this terminal protoplasmic material is essential for the formation of germ-cells, and that it has a formative

function. However, since these embryos were abnormal in other respects also, it is possible that other conditions are present in the centrifuged egg which interfere with the production of germ-cells at the "posterior end." Nevertheless, it is significant that the results agree essentially with those of Boveri ('10), who has shown in the eggs of Ascaris, by means of centrifuging, that certain protoplasmic regions of the egg determine which cells become germ-cells.

CHAPTER XXIII

ARTIFICIAL PARTHENOGENESIS

The development of eggs that have not been fertilized is an old and familiar story to zoölogists. In fact, eggs have sometimes been classified as of two kinds: those that are facultatively parthenogenetic, i.e., having the power to develop to a greater or lesser degree without fertilization; and eggs that are obligatorily parthenogenetic, i.e., that develop under normal conditions without being fertilized. A wider familiarity with eggs has shown in fact that to some degree practically all eggs may pass beyond the stage at which they are normally fertilized, if sperm fail to enter at that stage. Moreover, this stage is different in different eggs. It may appear, therefore, that a treatment that induces artificial parthenogenesis serves to help the egg over a critical period in its development rather than to add anything essentially new to its development.

The last statement may give an exaggerated idea of the situation, but it may, at least, serve to counteract a common idea that the artificial agents introduce into the egg something essential to its development that is lacking until supplied from outside. The figure of speech that expresses more nearly the point of view stated above—and most generalizations about artificial fertilization have up to the present time been little more than metaphors, even though expressed in quasi-chemical language—is that the ripe egg is like a machine, all ready to start a series of processes and that the start may be brought about in a considerable number of ways—in any way, in fact, that supplies the energy to set off the trigger-reaction. At any rate, as the sequel shows, the numerous attempts to discover the particular process essential to fertilization have signally failed.

Zoölogists have long been familiar with the fact that the eggs of some species will develop whether they are or are not fertilized. Aristotle (De Generatione Animalium) stated that the "bees

produce drones without copulation." Goedart in 1667 demonstrated artificial parthenogenesis in the moth, Orgyia gnastigma; Bonnet in 1745 discovered the virgin reproduction in the aphid; and Dzierzon's careful observations and experiments demonstrated in 1845 that the unfertilized eggs of the honey bee give rise to the drones. In more recent times many other kinds of animals have been added to the list, not only among ants, bees, wasps and other Hymenoptera and moths, but also in several other groups of animals (nematodes, starfish, trematodes, insects) and in plants.

In the early literature there are also several unsatisfactory references to the incitement of unfertilized eggs to parthenogenetic development by special treatment. These references were looked at somewhat askance until better methods demonstrated beyond question that unfertilized eggs could be aroused to continue their development. The early statements of Tichomiroff ('85, '02) that eggs from a virgin female silk-worm moth could be started to develop by bathing them in concentrated sulpuhuric acid for a few seconds or by rubbing them gently have been both doubted and confirmed. Later workers (Vernon '99, Quajat '05, Kellogg '07, Escaillon '16, '17, '18, Cavazza '24, Grandori '24, etc.), have on the whole obtained confirmatory results, but it has also been shown that certain races, without any treatment at all, produce eggs, many of which undergo changes comparable in some ways to early development. Unless carefully controlled material is used, the development of this egg after special treatment is open to the suspicion that the results may be due to a natural inclination to parthenogenesis rather than to artificial treatment.[1]

It was reserved for another line of work in later times to open up a new attack on the problem of parthenogenesis by methods that seemed at first to promise to show, not only how the exciting agents start development, but possibly even to furnish a clue as

[1] The first reports of parthenogenesis in the silk-worm moth were, according to von Siebold ('56), those of Constande Castellet (1795), of Herold (1838), P. de Filippi (1851) and Bousier (1847) von Siebold (1852) Schmidt (1854). How much credit can be given to the statement of Barthélémy (quoted by von Siebold 1871) that caterpillars sometimes emerge from unfertilized eggs of the silk-worm moth is difficult to say. It seems even less probable that Boursier's statement is true, that a female silk-worm, which had not paired with a male, after being placed in the sun, produced eggs that hatched into caterpillars. There can be no doubt however that other moths (Solenobia) regularly reproduce by parthenogenesis.

to what happens when normal fertilization takes place. These expectations arose from the results of Loeb's work in 1900,—results that Loeb and others carried much farther in the following years.

This work had been preceded by other promising observations in the same field. Mead's work ('96, '97) on the eggs of the annelid, Chaetopterus, treated with sodium chloride came first in point of time. When the normal eggs of Chaetopterus are removed from the body of the worm, the nucleus is still intact. If placed in sea water, the nuclear wall disappears and the first polar spindle is formed. Development then comes to a standstill. If a spermatozoön now enters the egg, the first polar body, and then the second is thrown off, and after the union of the two pronuclei cleavage proceeds. Mead found that when unfertilized eggs with polar spindles are placed in sea water to which one half to one per cent of sodium chloride is added, they proceed to form polar bodies as though fertilized. Indications of a first cleavage appear later with the characteristic protrusion of the yolk-lobe. Progress then ceases, but it was obvious that the first steps in development had been taken. Richard Hertwig ('96) found that when eggs of the sea-urchin are treated with strychnine, radiations appear around the nucleus, and the nucleus then undergoes changes like those preparatory to division.

Morgan ('96) found that "a normal or abnormal cleavage may take place" if unfertilized eggs of Arbacia are put into sea water, to which certain percentages of sodium chloride have been added, and are then returned to sea water. If left too long in the salt solution the eggs may pinch off balls but the latter process has no relation to the artificial asters that have been produced by the action of the salt. Three years later Morgan ('99) described in greater detail the action both of sodium and magnesium chloride on the unfertilized eggs of Arbacia. The main results were: (1) the cleavage of the unfertilized egg subjected for a short time to salt solutions; (2) the development of artificial asters, and their participation in the cleavage; (3) the development of a bipolar nuclear spindle with centrosomes at its poles. In the following year ('00) further work was carried out on the relation between the strength of the solutions and the time of exposure to them. It was pointed out that after exposure to a 5 per cent solution of magnesium chloride for five

minutes one-quarter of the eggs divided into 2 or 4 cells; for ten minutes one-third of the eggs divided; for twenty minutes most of the eggs divided; after thirty minutes half divided; and after sixty minutes none divided. It was found that weaker solutions require a longer time to produce an effect than stronger solutions, or conversely that it takes a shorter time for stronger solutions to produce a given result. The possible connection between the loss of water by the egg in the salt solution and the subsequent cleavage was discussed. "The fact that the egg shrinks in the salt solutions suggests that the cleavage may be connected with the loss of water of the egg." If so, some relation between the osmotic pressure of the solutions and the results might be expected "yet the result shows that the salts act in very different ways on the egg. Although there is ten times as much sodium chloride as magnesium chloride in sea water, yet the eggs are more easily injured by a small addition of the former salt. The shrinkage of the egg brought about by the solutions can hardly be the only cause of the phenomena." This problem has been much discussed in the later literature dealing with artificial parthenogenesis, and it still remains to be shown whether osmotic pressure alone or combined with simultaneous effects of a chemical nature is the essential factor in some of the methods used to induce cleavage of the egg.

Morgan ('00) studied the internal changes produced by magnesium chloride on unfertilized eggs of the sea-urchin (Arbacia), and found that eggs treated at various intervals and by varying amounts of the salt undergo mitotic divisions. The cleavage of the eggs was studied in detail as well as the cleavage of the nucleus, and the method of development of the cytasters by which the division is accomplished. It was shown by varying the strength of the solution and the time of exposure that the egg not only divides into two or more cells, but that once started the division continues for some time until a number of cells are formed. In none of the cases observed was the cleavage pattern strictly normal. The blastula-like embryo that resulted did not develop further. Owing to the absence of conformity to the regular type of cleavage of the sea-urchin's egg and to the failure to produce later embryonic stages, it was not at the time realized that with very slight changes in the time of exposure such later stages might be obtained.

By the same method Loeb ('00) induced many of the sea-urchin eggs treated with magnesium chloride to proceed to later stages, even to early pluteus stages.[2]

Loeb had obtained in the summer of 1899 swimming plutei from unfertilized sea-urchin eggs that had been kept in a mixture of sea water and magnesium chloride solution (50 cc. sea water plus 50 cc. of 2½ normal $MgCl_2$) for 1½ to 2 hours and then returned to sea water. In the course of the following winter at Pacific Grove, California, he found not only that $MgCl_2$, but that sodium chloride or even sugar added to sea water incited cleavage and development, but the results were not so regular as before. In the summer of 1900 Loeb showed that $MgCl_2$ does not have a specific action, and that it is immaterial whether the result is caused by electrolytes like $MgCl_2$, NaCl, KCl, or $CaCl_2$, or by the addition of non-electrolytes, such as cane sugar and urea. The earlier partial failure at Pacific Grove "was due to the fact that the solutions of salts . . . were not isosmotic." A slight increase in the osmotic pressure of sea water was found sufficient to start development although the "development never goes farther than the blastula stage, and as a rule, not even this far." Loeb also noticed that the eggs in the hypertonic solution lose water, which is taken up again on the return to sea water, and since the eggs sometimes segmented while in the hypertonic solution, he concluded at that time that the loss of water and not its subsequent replacement is the cause of the segmentation. That they developed no further if kept in the salt solution was supposed to

[2] Loeb had already in 1892 studied the effect on the fertilized eggs of Arbacia of adding 2 per cent sodium chloride to sea water. The development of the eggs is suppressed, but if after three or four hours they are returned to sea water they will segment at once. The obvious implication of the paper was that the nuclei continued to divide in the hypertonic solution, but not the cytoplasm. Morgan repeated the experiment in 1894 and showed that the result was probably due to the development of artificial asters in the cytoplasm which had not been observed by Loeb, or had been mistaken for nuclei. Norman ('96) made again the experiment and confirmed the observation as to the development of the artificial asters. He also showed that in *solutions of certain strengths* multipolar mitotic figures develop which on return to sea water may produce several nuclei that may take some part in the subsequent division. The account by no means confirms the earlier conclusions of Loeb's in regard to the action of the hypertonic solution. (See Loeb's account in "Artificial Parthenogensis and Fertilization" 1913, pages 50–51.)

be due to the harmful effect of too long exposure to the solution. Bataillon in 1900 induced cleavage in the frog's eggs by treating them with salt solutions and with sugar. He attributed the result to parthenogenetic development, and since salt and sugar give similar results he concluded that the increased osmotic pressure of the medium was the most influential factor in starting parthenogenetic development, which is one of the conclusions at which Loeb had arrived at about that time.[3]

Since the embryos produced by hypertonic solutions never came to the surface as do normal embryos, and since they "never formed a characteristic fertilization membrane," Loeb ('05) sought for a better method. He found that unfertilized sea-urchin eggs placed for a couple of minutes in sea water, to which a little ethyl acetate had been added, formed a typical membrane and began to divide when returned to sea water. They did not, however, develop into larvae. If, on the other hand, eggs were first exposed for two hours to hypertonic sea water and were next treated with sea water plus ethyl acetate and then removed to sea water, many of the eggs developed in quite a normal manner. It was found that any monobasic fatty acid (formic, acetic, proprionic, butyric, valerianic) induced membrane formation. Carbonic acid behaves like the high fatty acids (e.g. heptylic acid).

Loeb reached the conclusion from the following experiments that membrane formation is the deciding condition for development. Unfertilized eggs were placed from 1½ to 2½ minutes in 50 cc. sea water plus 2.8 cc. N/10 monobasic fatty acid. All the eggs produced membranes. But if the eggs are removed a little too soon from the acid, only some of them form membranes when returned to sea water. If then treated with hypertonic sea water and afterwards put into sea water, only such eggs as have formed membranes develop. "Membrane formation is, therefore, the deciding condition for development." As will be shown later this significance of membrane formation has been challenged by later workers.

Further proof of his conclusion Loeb found in another experiment. Any substance that causes haemolysis also calls forth membrane formation, such as saponin, bile salts, ether, hydrocarbons, etc. No matter by what means membrane formation

[3] See Bataillon 1900 and 1912 (review), also Loeb's book on Artificial Fertilization.

is induced, it starts development of the egg, if afterwards the eggs are treated with the hypertonic solution.

One further experiment may be cited. It had been shown that, after the acid treatment alone, the cleavage spindle may form, and the eggs may even segment, but the eggs then disintegrate. It appears then that "membrane formation does indeed initiate development, but that it leaves the egg in a condition in which cell-division becomes fatal to it." If, however, eggs that have been in the butyric acid are put into sea water plus KCN (50 cc. sea water plus 2 cc. $\frac{1}{20}$ of KCN) for three hours, some of the eggs develop into larvae. "In all these experiments the perfect regularity of the segmentation was very striking; the segmentation is almost as good as in eggs fertilized by sperm." The larvae come to the surface and large numbers of plutei are produced. Loeb explains these results as follows: "Membrane formation sets going (accelerates) the chemical reactions which determine development. If we interrupt the oxidations in such an egg there will accumulate in it decomposition products which ought to be removed by oxidations. I suppose that this accumulation of decomposition products leads to the injury of the egg, which is shown by the fact that the longer the eggs remain in the cyanide solution the more their vitality is impaired"; for, eggs that had been left in the cyanide solution only two or three hours after membrane formation, produced more plutei than did eggs left longer. The cyanide acts as a corrective to the cytolytic process induced by any agent that causes membrane formation. The experiment shows, Loeb concluded, that "the artificial membrane formation starts the development of the egg, but that it leaves it in a sickly condition, which causes it to disintegrate rapidly at room temperature. In order to make such eggs normal, they must undergo a second treatment: they must either be exposed for a short time to a hypertonic solution, or for a somewhat longer time to a normal sea water solution in which oxidations of the egg are suppressed." As will be shown later, other workers, who have studied the internal changes induced by these artificial media, have reached a different conclusion as to the action of the second so-called corrective agent.

Loeb found that certain bases are also capable of inciting development. The eggs must be treated much longer with a base

than with an acid. The bases do not cause (except rarely) a true membrane. A fine gelatinous layer appears over the egg. If such eggs are removed after 25 minutes and placed in hypertonic sea water for 15 minutes, then put into sea water, a large number of eggs develop into larvae, many of which are normal. Only those eggs that showed a gelatinous film developed, and this film did not usually appear in the first solution but later in the hypertonic sea water. Weak bases such as NH_4OH are more effective than strong bases such as NaOH or KOH. Other weak bases, such as the amines, are also effective. One of these, protamine "prepared from the sperm of salmon, is one of the most efficient substances for the causation of artificial parthenogenesis."

The weaker bases are more efficient than the stronger ones, because the weaker bases diffuse more rapidly into the egg, while the stronger bases do not diffuse at all or only to a slight extent. Harvey ('10) has shown that this is true not only for eggs, but for other kinds of cells also.

There has been some discussion concerning the nature of the cytolysis, observed in eggs left in solutions that cause membrane formation. At one time ('08) Loeb referred the formation of the fertilization membrane to a liquefaction of the lipoid on the surface of the egg. Protoplasm, according to von Knaffl-Lenz ('08), is rich in lipoids and any solution that liquefies these, will cause cytolysis of the protoplasm, because the egg can only swell (or take up water) when the "condition of aggregation of the lipoid" is altered. Other writers have, however, brought forward evidence unfavorable to this interpretation of membrane formation, and Loeb also has pointed out that the fertilization membrane itself is insoluble in benzol or in any other lipoid solvent.

Loeb found ('07, '08) that the blood and tissue extracts of other animals will cause the unfertilized eggs of the sea-urchin to form a fertilization membrane.[4] If, then, the egg is next treated with a hypertonic solution it will develop. On the other hand, if unfertilized eggs are treated with the blood or tissue-extracts of their own species they will not form fertilization membranes.

Even the blood of mammals (dog, frog, ox) may cause mem-

[4] Whether this is a true membrane or only a swelling, has been questioned. (See Heilbrunn.)

brane formation on the sea-urchin's egg. Not all eggs respond to the treatment, only those of certain females, but even those that do not do so at first will respond if they are first sensitized by the addition of a little strontium chloride to the solution. Loeb thought that the failure of the blood of the same species to act on its own eggs is due to the fact that foreign lysins can penetrate into the egg, while those of the same species are unable to do so. For, in general, only those substances that diffuse are efficient agents. Watery extracts of the sperm of foreign species cause membrane formation, but a watery solution of the sperm of the same species does not. This he interpreted to mean that while foreign lysins may diffuse into the egg, its own lysins cannot do so, but must be brought in by the penetration of the living sperm.

Loeb attempted to bring these results into line with certain experiments where concentrated sperm of foreign species may cause membrane formation. Kupelwieser ('06, '09) showed that very concentrated sperm of the mollusc, Mytilus, may cause typical fertilization membranes around the eggs of the sea-urchin. If such eggs are then treated in the usual way with hypertonic sea water they will begin to develop. Loeb has shown that filtered sperm of Chiton, starfish, and other sea-urchins has a like effect. These results were all explained by Loeb as due to the action of the foreign lysins present in the sperm-suspensions. An interesting point in this connection is that the living sperm of a shark, or starfish do not produce membranes on the sea-urchin's eggs, but if extracts of the same sperm are used the membrane is formed. It was suggested that in the latter case, the lysins are extracted from the sperm and may diffuse and are "in higher concentration than if a single spermatozoön of a foreign species" reaches the egg.

The extensive work that Loeb carried out in the field of artificial parthenogenesis served as a stimulus that has attracted a number of other investigators, who have used in the main the methods worked out by Loeb, the best of which produce larvae that resemble normal larvae in every detail. Some of these larvae are probably haploid, but it is doubtful if haploid sea-urchin larvae can become normal adults. The work of Delage, and of Shearer, de Morgan and Fuchs shows, it is true, that the adult form may also be attained; but there still remains the

possibility that the few recorded cases of this kind may be cases where a doubling of the chromosomes has first taken place, and if so the larvae are diploid.

Loeb's attack on the problem was a direct attempt to discover the nature of the chemical changes induced in the egg by the stimulating agent. The results show that the effect may be induced by chemical means, a view that is now generally accepted, but two general criticisms of this conclusion have been made. First, the actual chemistry involved is necessarily more often inferred than proven. The agents may only serve to start a chain of unknown physical or chemical processes whose nature is obscure. Second, it has been shown that a single reagent may suffice to produce results as successfully as those brought about by the double method.

Delage ('08) recommended a solution of sugar, tannin and ammonia in sea water as a receipt for producing artificial parthenogenesis in the eggs of Strongylocentrotus lividus.[5] It was later pointed out by Lloyd ('14) that this solution is not isotonic with sea water as Delage supposed but hypertonic; that the tannin was little if at all soluble in the solution; and that more NH_4OH is added than sufficient to neutralize the tannic acid. Later work has shown that equally good results are obtainable when the tannin is left out. It is probable, therefore, that the parthenogenesis was brought about by an alkaline sugar solution. Delage has suggested that development is initiated by alternate coagulation and liquefaction of the cytoplasm, a view that has found some support in later work, at least to the extent that such changes do take place during cleavage, but aside from the fact that hypertonic solutions may induce some coagulation at first it is not obvious how this initiation could lead to the successive alternations of coagulation and liquefaction that take place at and between consecutive divisions. The other method discovered by Delage for causing parthenogenesis in starfish egg, viz., by the use of CO_2, may be appealed to in support of his idea of an initial coagulation.

Shearer and Lloyd ('13) have used various modifications of Loeb's method to bring about artificial parthenogenesis in the egg of Echinus esculentus at Plymouth. They recommended

[5] Sugar 388 gr. in 1 liter distilled water. To this 700 to 300 cc. sterilized sea water is added + 0.15 tannin + 3 cc. of ammonia.

making the sea water slightly alkaline. They have also tried out Delage's methods and various combinations of these with Loeb's improved method. With the new methods of rearing echinoderm larvae worked out by Allen and Nelson ('10), Shearer and Lloyd have tried to carry parthenogenetic larvae through to adult stages. While they have succeeded in getting some plutei through the metamorphosis they have not carried them much beyond that point. They state, contrary to Delage, that the artificial plutei and the metamorphosing plutei differ in certain characteristic ways from those of the normally fertilized egg. Possibly they are haploid but the authors furnish no data on this point.

It has been shown by Just ('22) that normal top-swimming plutei of Arbacia can be produced as successfully by the use of hypertonic solutions alone as by the use of the double method of butyric acid followed by hypertonic solution. Success depends on the exact proportions of the salt solution and the sea water and on the length of exposure. All eggs from one female behave in much the same way, but the eggs of different females may require slightly different treatments to give the optimum results. Therefore it is better to subject portions of the eggs of each female to a series of solutions at and on each side of the supposed optimum. A hypertonic solution made up with 20, 22, or 24 parts of 2½ M NaCl (or KCl) plus 80, 78 or 76 parts sea water causes all the eggs to lift off membranes *while in the solution.* This happens from 15 seconds to 5 or 10 minutes according to the strength of the solution employed. The membranes lift off as a rule more slowly than from normally inseminated eggs, but they are as clear and possess as wide a perivitelline space as normally fertilized eggs. Those sets that produce the clearest and most typical membranes are the ones that, when the eggs are returned to sea water, produce the highest percentage of plutei. These come to the top in large numbers and behave in every respect like the plutei from normally inseminated eggs. Just states that the results are in every way equal to those produced by the double method of butyric acid followed by hypertonic sea water. He argues that since the hypertonic sea water is capable of producing membrane separation comparable to that of normal fertilization, and since this leads to the development of normal plutei, there is no necessity to assume, as Loeb does,

that a corrective is required to stop the cytolysis induced by the initial stimulus to development. It may be true, nevertheless, that if the acid treatment is not as carefully controlled as in Just's experiments, the second treatment may serve in the way postulated by Loeb.

Cytological Changes in Eggs Incited to Parthenogenetic Development

The cytological changes induced by several of these methods have been studied by several workers. (Morgan '94, '96, '00; E. B. Wilson '01; Delage '08; Just '22; Herlant '12, '13, '14; Fry '25 and Tharaldsen '26.)

Wilson ('01) studied the effects of the magnesium solution on the interior of the egg using Loeb's receipt of equal volumes of sea water and a 12 per cent solution of magnesium chloride. He found that unfertilized eggs of Toxopneustes give rise "to swimming blastulae and gastrulae and in many cases plutei. None of these in my experience were exactly like those arising from fertilized eggs. The cleavage is sometimes nearly regular, but more often differs widely from the normal type showing many strange forms similar to those described by Morgan and Loeb." The blastulae are distinguishable "at the first glance" from the normal ones, the gastrulae often approach nearly to the normal forms, but frequently have thicker walls and a greater number of mesenchyme cells. The plutei are often distorted and even monstrous in form "but a considerable portion of them approach nearly to those arising from fertilized eggs." None, however, were normal in every respect. This account by an experienced embryologist gave for the first time a true picture of the embryos resulting from the method of artificial fertilization that had up to that time been used. Wilson also determined that the eggs divided with the haploid number (18) of chromosomes. In the light of later work it may appear that some at least of these abnormalities may be due to the haploid condition of these embryos; for, abnormality is not an uncommon feature of animal types that have been caused to develop with the half number of chromosomes.

Seventeen years later Herlant ('18, '19) studied the eggs of the sea-urchin, Paracentrotus lividus, whose transparency makes it possible to see something of the changes taking place

in living eggs artificially fertilized, and thus give a correct seriation of the stages. The eggs were subsequently also studied by means of sections. The difference in his results, Herlant says, from those of his predecessors is "principally because these authors have failed to realize the importance of a methodical study of the living eggs."

He finds that the only apparent modification that takes place during a short preliminary treatment with butyric acid is the dissolution of the chorion (the outer jelly). If the eggs have

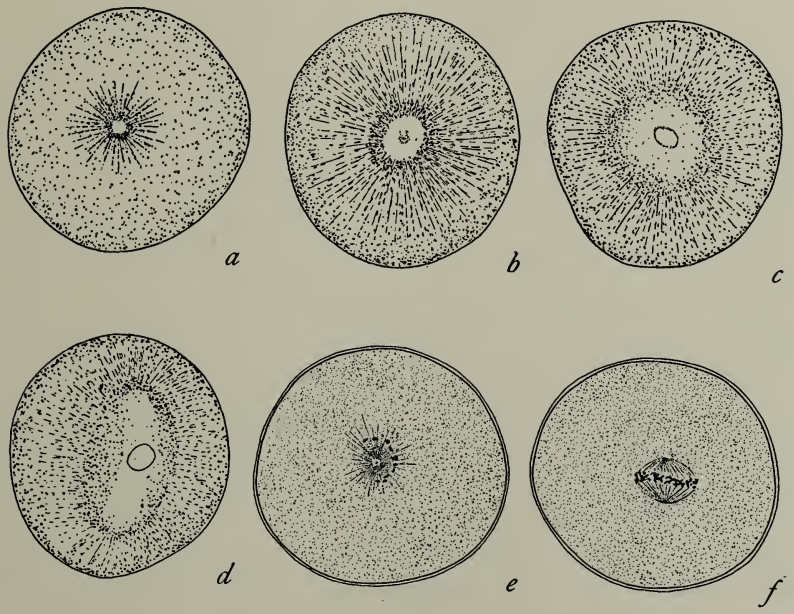


FIG. 242.—Sections of egg of sea-urchin, Paracentrotus lividus, that have been artificially fertilized by single treatment. (After Herlant.)

received only the acid treatment and are then returned to sea water, the nuclear wall is dissolved 1 hour and 45 minutes after the treatment. At this time there is formed in the vicinity of the nucleus a slight condensation of the cytoplasm around which there appears an irradiation that rapidly extends outwards (Fig. 242*a*, *b*). As this monaster increases in size, several zones may be found in it. After 40 minutes it reaches its greatest development. Then about 2 hours and 40 minutes after activation the center of the monaster loses its rays, which now extend only

from the outer zone to the periphery of the egg (Fig. 242*c*), and the clear zone, instead of enlarging expands lengthwise and assumes the form shown in Fig. 242*d*.

These changes mark the end of the first monaster and the reconstitution of the nucleus. At about this time a change takes place in the surface (as noted by Boveri '03, and by Painter '18). The egg, spherical until now, assumes an irregular contour and grooves appear in its surface. They represent an abortive attempt to divide.

Ten to twenty minutes later, the egg becomes spherical again. Three hours after activation the egg resumes the appearance that it had when the monaster first appeared. This period of repose lasts 30 minutes. Then the same series of events repeats itself; the nucleus dissolves and the cytaster enlarges. The second period of activity is shorter than the first (30 instead of 50 minutes). The second period of repose is also shorter (20 instead of 30 minutes). A third, fourth, fifth, and sometimes even a sixth cycle of similar events follows. The nucleus gradually enlarges at each period of activity, the chromosomes having divided. In the fifth and sixth cycles the radiation becomes very faint. The nucleus may become as large as the ovarian nucleus.

After a time the eggs show signs of disintegration—a sort of cytolysis setting in. Herlant concludes that "the simple activation of the egg of the sea-urchin is characterized essentially by the formation of a monaster that repeats itself rhythmically without ever provoking the segmentation. It is only after numerous attempts to divide that the egg begins to destroy itself by cytolysis. This appears only as the final result of the incompatible cytological conditions that are produced and has nothing to do with the cause of the preceding events." [6]

Eggs that have received Loeb's double treatment were also studied both alive and after being sectioned. The eggs were activated by butyric acid, put into sea water for 20 minutes, and then transferred for 30 minutes to a hypertonic solution.

The last solution causes the eggs to become somewhat opaque due to dehydration. The volume of the nucleus remains the same until it begins its growth cycle. When removed from the hypertonic solution, the egg recovers little by little the aspect

[6] It should be noted that Just has shown in Echinarachnius that complete activation may be brought about with normal larvae by sodium chloride alone.

of an egg when activated only by the acid; its protoplasm absorbs water, becomes transparent, and the nucleus begins to enlarge.

An aster develops near the nucleus but it is not the only one; for, appearing at the same time, or a little later, a variable number of accessory asters appear scattered throughout the protoplasm. These are the "artificial asters" or cytasters described by Morgan ('00) and by Wilson ('01). When the solutions have been suitably applied, not more than one to three accessory asters appear. Their rays grow rapidly, converging

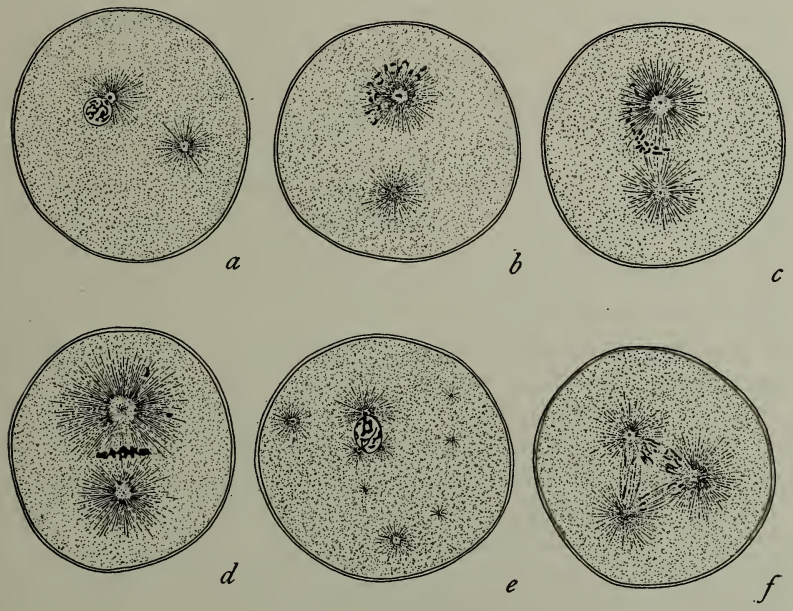


FIG. 243.—Sections of egg of sea-urchin, Paracentrotus, that have been artificially fertilized by the double method. (After Herlant.)

in a brilliant granulation in the center of the aster. The center enlarges as the asters grow, but the accessory asters are always smaller than the perinuclear or central monaster.

The simplest cases are those when only one accessory aster is present. For a time it remains independent of the central nuclear aster (Fig. 243*a*), but as the rays of each extend they touch each other, and there is established a liaison between the two asters. Together they form a bipolar mitotic figure that does not differ from a normal one except that one aster is larger

than the other. Thus the monaster near the nucleus combines with the accessory aster to produce a bipolar figure (Fig. 243*c*, *d*).

The chromosomes that have now appeared become arranged at the equator of the spindle where they divide and migrate to the poles (Fig. 243*d*). Segmentation of the egg takes place about three hours after activation (15 degrees C.). The first two blastomeres are generally unequal, corresponding to the relative size of the two polar asters.

Often two, or three, or rarely more cytasters appear. Two or three of them may then establish spindle connections with the central aster (Fig. 243*e*, *f*). Sometimes one, or two, or three remain outside the spindle. When a triaster forms, the egg divides into three cells; when a tetraster, it divides into four cells. Owing, no doubt, to the irregular distribution of the chromosomes in such cases, these eggs fail to produce normal embryos. When only one accessory aster connects with the central aster, and one other accessory aster is present, the first cleavage may divide the egg into two cells, one of which contains also the extra accessory aster.

The not infrequent irregularity in the size of the first two blastomeres appears to have no injurious effect on development, since it is equalized in later divisions. The larger cell contains the central aster, that has every appearance of being the same kind of aster as that which develops after simple treatment with acid, but in the latter case, as stated above, it fails to bring about division, and although it appears and reappears several times it fails to divide and later disappears. But if the central aster has established connection with an accessory aster it goes to one cell and in that cell it subsequently divides and its division products in daughter cells also divide. Its behavior appears to be determined by the connection it makes with a cytaster. On the other hand, the difference in its fate may be more probably ascribed to the second treatment it receives in the hypertonic solution.

In cases where an accessory aster becomes included in one of the first blastomeres along with a nucleus and its aster, it may later establish connections with the spindle and cause irregularities in division with consequent abnormalities.

Herlant concludes that the most important factor, the one

comparable with normal fertilization, is the treatment with the butyric acid. It starts the development, but it alone fails to cause the segmentation because there is only one aster. The second treatment does not change the process set up by the first reagent, but superimposes upon it another element, namely, the accessory aster that insures the segmentation—"the second factor does not correspond with any of the normal phenomena." The statement obviously goes too far, for it had been shown by several earlier workers that, by means of the hypertonic solution alone, asters may appear that bring about cleavage and even normal development may follow. The distinction that Herlant draws, appears therefore, incorrect.

Herlant studied the relation between the time intervals, and the success of the treatment in the following way. The eggs were activated by butyric acid, then placed every five minutes in the hypertonic solution (50 cc. sea water plus 8 cc. "5 de solution" 2½ M. of NaCl). Each lot remained for the same length of time in this solution and was then placed in sea water. After an hour and a half, when the cytasters were present and the eggs about to divide, "the number of cytasters present was recorded." In the first lots, no asters were present. Their number increased from lot to lot up to the time of those treated 30 minutes after activation. After this they decreased down to the 50 minute lot. The segmentation in these eggs corresponded closely with the number of cytasters present. Those without cytasters did not segment. On the other hand, too many asters interfered with cleavage and resulted in cytolysis. The formation of blastulae was best accomplished in those sets in which fewer asters were present. Too many asters are injurious for normal development.

If normally *fertilized* eggs are treated with hypertonic solutions for different lengths of time it is found that only those develop into normal blastulae in which no accessory asters have appeared. In other words, the normal machinery for division is interfered with in proportion to the formation of accessory asters in such eggs. Comparisons between hypertonic solutions of different strengths show that too weak and too strong solutions are inefficient and their failure accords with the observed absence, in both, of cytasters.

Herlant found, for the species he studied, that NaCl and KCl are necessary in the hypertonic solution in order to produce

cytasters. The two salts, $CaCl_2$ and $MgCl_2$, are useless, and if in excess, inhibit the formation of asters even when the other two are present. It may be recalled that while $MgCl_2$ has been found capable of producing asters in the American species, Arbacia, etc. (Loeb, Morgan, Wilson), Just ('22) has had his best success with this same species of Arbacia by using NaCl or KCl hypertonic solutions.

Loeb had shown that an alkaline hypertonic solution is more efficacious than a neutral or acid solution. Herlant found that an alkaline solution favors the formation of cytasters. He also found, as had R. S. Lillie earlier, that potassium cyanide, ether, chloral hydrate, and alcohol, even in very small quantities, suppress the formation of asters and prevent cleavage.

More interesting are Herlant's results that relate to permeability. Eggs were activated by butyric acid (or else activated by normal fertilization), and transferred every five minutes to a hypertonic solution. They were left there for 45 to 60 minutes, then examined. It was found that only those eggs are plasmolyzed that have been treated by the hypertonic solution within a certain time after activation (40 to 50 minutes). This result shows that while the fertilization membrane is permeable at the beginning, it changes slowly and becomes semi-permeable and finally impermeable. This is made more striking by using solutions so strong that the membrane is made impermeable. Then no cytolysis results as it would under other circumstances. The following table (Table XXVI) makes this evident.

"The egg is capable of forming accessory asters only if it receives the hypertonic treatment during the period of development when the membrane is permeable to the salt and the asters are formed in proportion as the permeability is greater." It follows that the hypertonic solution is active only when the salt penetrates the egg. It is the presence of the salt in the egg and not the dehydration of the egg that is the significant factor in artificial parthenogenesis.

This conclusion can readily be verified, for, if the production of cytasters depends on the permeability, those reagents that increase or diminish the permeability should augment or diminish the production of cytasters. This relation Herlant ('18) found to hold. NaCl and KCl augment, while $CaCl_2$ and $MgCl_2$ diminish the permeability (results that agree with those

TABLE XXVI

	Minutes After Activation	Eggs Cytolyzed, Per Cent	Eggs Plasmolyzed, Per Cent
1	5	10	5
2	10	50	0
3	15	75	0
4	20	100	0
5	25	100	0
6	30	80	20
7	35	30	50
8	40	10	80
9	45	10	80
10	50	2	85
11	55	0	100
12	60	0	100
13	65	0	100
14	70	0	100
15	75	0	100
16	80	0	100

of R. S. Lillie, Osterhout, Clowes, McClendon, Brooks). Now the two former salts favor cytaster formation, the latter tend to prevent it. Herlant also showed that the OH-ions augment the permeability and also cytaster formation. The H-ions decrease permeability and suppress or weaken cytaster formation. Anaesthetics also suppress aster formation.

Herlant's general conclusion is that the salts produce the cytasters by penetrating into the cell. What then occurs is obscure, but Herlant offers the following suggestions. "The development of the aster is a phenomenon of coagulation (gel), its disappearance is due to liquefaction (sol). Protoplasm is a reversible gel as suggested by Fischer and Oswald ('05), Delage ('08), Kite ('13), Chambers ('17), Heilbrunn ('15) and others. The aster is produced at the time that the cell becomes permeable to water and salts. It is probable that the irruption does not take place without producing profound modification in the physico-chemical constitution of the colloidal protoplasm and it is difficult not to believe that these elements take a direct part in the formation of the aster."

More recently Fry ('25) has examined in carefully controlled observations the origin of the mitotic figures in the egg of the

sand dollar (Echinarachnius parma), and has shown that those parthenogenetic eggs, that approach most nearly the normal type of cleavage and produce the best embryos, belong to the type whose mitotic figure arises by division of the aster near the nucleus (nuclear aster) and not, as Herlant supposed, by a combination of a central aster (monaster) and a peripheral one. A similar conclusion has been reached by Tharaldsen in regard to starfish eggs, where, however, the internal conditions at the time of treatment are somewhat different from those in the sea-urchin (see below). Fry studied both living and prepared material, and especially the history of individual eggs that were isolated. He also showed that the latter result is in strict accord with the mass results. He found many eggs of the type described by Herlant, but proved that such eggs give rise at most to a very small percentage of embryos. The latest and most approved method of treatment for this egg (that worked out by Just '19) was used, namely the combination method of butyric acid solution (2 cc. $\frac{1}{10}$ normal butyric acid plus 50 cc. sea water) for about 35 minutes, then sea water, where the membranes are lifted off (25 minutes), followed by hypertonic solution of sodium chloride (5 cc. 2.5n NaCl + 50 cc. sea water).

In Fig. 244, the normal development of the one aster eggs is illustrated. Their development is said to be markedly similar to the normally fertilized eggs and shows equally a contrast with corresponding stages of artificially activated eggs that contain additional cytasters. The results confirm the earlier ones of Wilson ('01), namely, that the mitotic figure arises from the division of a single nuclear aster. (See also Hindle '10, and Chambers '21.)

Fry found also that two aster eggs having a nuclear aster and a single cytaster do not develop normally—most of them perish as irregular blastulae, while a very few produce gastrulae of partial size. "That the two aster egg is the first type of response to cleave is probably one reason why Herlant selected it as the normal type of development."

A further question may arise as to the sharp contrast that is drawn in Fry's work between the nuclear aster, so called, and the other asters lying farther away that are called cytasters. The latter rarely divide according to Fry. They may, however, form combinations—mitotic figures—with the nuclear aster if

they lie near to it, and the chromosomes may become arranged on a central spindle that develops between them, as earlier observers (Morgan, Wilson) have recorded. The only apparent difference, then, between the nuclear aster and the cytasters is in its position and in its greater readiness to divide. This may

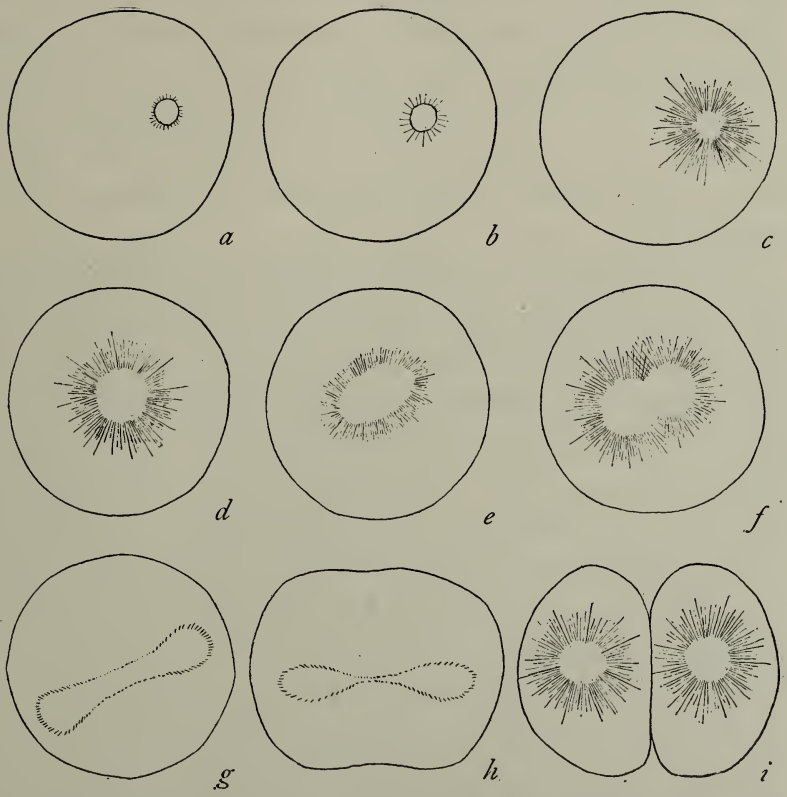


FIG. 244.—Optical sections of living egg of sea-urchin, Paracentrotus, after artificial fertilization, showing the formation of a single large aster that divides to form the bipolar division-figure. (After Fry.)

mean that the material lying near the nucleus responds more readily to treatment, and the aster that is produced there is larger. Possibly the synchronous changes in the nucleus itself are contributory to the division. That the nuclear aster is not essentially different from the other asters is evident (1) from their ability to combine with it and produce multipolar mitotic figures, and (2) from the migration of the chromosomes to all

the poles of these figures. The failure of these types (like those of Herlant) to produce normal embryos as frequently as the single aster types may, I suspect, be explained in part by the more frequent irregularity in the distribution of the chromosomes.

Fry also studied the development of cytasters in fragments cut from the egg. In this egg, as in other sea-urchins, the polar bodies have been given off some time before the operation, so that the nuclear sap in the original egg-nucleus has already been set free, and may be assumed to be present in the fragments. Asters develop in the same way in the non-nucleated fragments as in whole eggs, when treated by the same artificial reagents. They do not divide, and as a rule the fragments rarely divide, and then irregularly.

The conclusions that Herlant reached concerning the difference between single and double treatment have not been confirmed by Tharaldsen for the starfish egg (Asterias forbesii). The eggs were activated either by treatment with butyric acid (N/126) alone (as recommended by R. S. Lillie), or by Loeb's double method of butyric acid followed by hypertonic sea water. Either method will give complete development. The eggs were exposed (39 minutes after removal from the ovary, at which time the wall of the egg-nucleus was disappearing) to the butyric acid for eight minutes, and then transferred to sea water. When the double method was used, the eggs were left for four minutes in the butyric acid, transferred to sea water (18 min.) and placed in the hypertonic solution (250 cc. sea water plus 30 cc. 2.5 M. NaCl) for twenty minutes; finally to sea water again.

The stimulus supplied to the eggs suffices, if successful, to cause them to give off both polar bodies, and, then, without delay to produce the first cleavage spindle. According to Tharaldsen's observations of the different types of eggs that appear after treatment, the only one that gives rise to normal larvae is that in which only one aster is concerned in the formation of the first (and second) polar body. In such eggs only a single aster appears later near the egg-pronucleus, and this divides to form the amphiaster of first cleavage.

The sea-urchin's egg and that of the starfish are in different "stages" when the artificial reagents are applied, and cannot be strictly compared. For instance, the eggs of the starfish were exposed to the solutions at a time when the normal polar spindle

was about to form. It would have formed without the stimulating agent and the second polar spindle also, but the egg would then have come to rest. After stimulation, however, the development proceeded, the second polar body was extruded (without further stimulation), and the segmentation spindle arose from a central aster that divided to form the spindle. In this instance, then, there could be no question of accessory asters taking part in the development since these were at the time on the wane. In the sea-urchin, however, they arose at the same time as the central aster and from the same immediate cause.

Artificial Parthenogenesis in the Starfish

Many methods have been tried to bring about artificial parthenogenesis of the eggs of starfish. In general it may be said that the methods that are successful with sea-urchin eggs give very poor results with starfish eggs, although Delage got good results with NaCl,[7] and R. S. Lillie with butyric acid. Conversely, two of the most successful methods for starfish eggs (carbon dioxide and mechanical agitation) give almost no results with sea-urchin eggs.

Delage's procedure is as follows. The eggs of Asterias glacialis are gently shaken out of the excised ovary into sea water. The egg still contains the large ovarian nucleus. Development then proceeds and the first polar body is extruded. At the time of its formation the eggs are transferred to sea water charged with CO_2. This reagent is most readily obtained from a sparklet bottle filled with sea water and charged from a "bomb" of CO_2. The CO_2 sea water is squirted into a dish and allowed to stand until the bubbles of CO_2 have been set free. The eggs are then pipetted into the solution, where they remain for about an hour. Five minutes are sufficient, but the results are better with a longer sojourn in the solution. The eggs withstand even 1½ to 2 hours submersion, but give no better results. All development comes to a standstill in the charged water. When the eggs are transferred to sea water, they slowly begin their development again. In about 1½ hours the fertilization membrane

[7] In one case 95 per cent of the eggs treated with NaCl after the first polar body had been formed developed as far as the "blastula" stage. $MgCl_2$ gave at best only a few cleavages.

appears. The first polar body is given off; but the second one is not formed in the majority of cases. After 3 hours the segmentation begins. A furrow appears on the surface and cuts the egg in two; each blastomere is seen to contain a nucleus. The segmentation process continuing, leads to the transparent blastula stage. The blastulae soon begin to swim. They gastrulate and become normal larvae, which reach in 5 days the auricula stage. If the eggs are in good condition 100 per cent of them segment. More of the treated eggs generally segment and form larvae than do untreated eggs when fertilized with sperm, of which as a rule only 30 to 40 per cent form larvae. It is important to note that with the CO_2 method only those eggs develop that have reached the first polar body stage. Eggs, whose nuclei are still intact when put into CO_2 solution, never develop further. If the eggs are allowed to proceed until the second polar spindle is formed the results are not so good, although, according to Delage, there is a short period, just as the second polar body is about to be given off, when the eggs, if treated with CO_2, will again give good results, but the outcome is not so good as when they are treated during the first polar body period. The presence of oxygen in the CO_2 solution is not necessary; for, eggs treated in this way give the same results as when oxygen is present.

The diploid number of chromosomes in Asterias glacialis is erroneously given as 18 by Delage. He found 18 in the blastomeres of treated eggs. The full number of chromosomes he supposed to be due to the suppression of the second polar spindle; and he thought the chromosome number was not reduced, because he supposed this occurred when the second polar body was given off.

Mathews ('01) has shown that development of the starfish egg (Asterias forbesii) may be brought about by agitation. The eggs are allowed to ripen in sea water for from 2 to 4 hours until both polar bodies are given off. The egg pronucleus is then reformed, and has reached a "considerable size." The eggs are placed in a test tube partly filled with sea water and shaken vigorously five or six times. They are then placed in sea water. The amount of agitation necessary depends on the age of the eggs. "After from 4 to 6 hours in the water the mere transference of the eggs from one dish to another by a pipette, or the jar caused by setting the dish containing the eggs down sharply on

the table is sufficient to start development in a small portion of the eggs with the result that swimming blastulae appear by morning."

The fertilization membrane appears a few minutes after shaking. The eggs change from a spherical shape to that of a flattened ellipsoid, the flattening beginning at the pole. The nuclear membrane fades within a few minutes, or may not disappear for several hours. Very little is said by Mathews about the cleavage of the eggs. Only irregular cleavages are spoken of. The egg generally divides into several cells at once. It is not probable that normal embryos ever develop from such eggs, and the term blastula stage does not imply a normal embryo. The few eggs that do produce embryos must be supposed to divide more regularly, but no such eggs are specifically described. It is stated that from less than 1 per cent to more than 50 per cent develop into swimming blastulae. Delage never obtained more than 50 per cent by his method. What percentage of these form normal larvae is unknown; in one case 30 swimming gastrulae in 4000 eggs are recorded; 75 "blastulae" and gastrulae in 2500 eggs in another case; and in other cases still fewer "swimming embryos" were found.[8]

Buchner ('11) has studied the changes that take place in the unfertilized eggs of Asterias glacialis treated by Delage's CO_2 method. After treatment with CO_2, and after the return to sea water, the first spindle was found. Very slowly the first polar body spindle passes through its division stages leading to an outer group of polar chromosomes and an inner group. The former may be extruded in a polar body that is pinched off from the surface in typical fashion, but frequently the chromosomes become vesiculated near the periphery, and come to lie in a flattened mass of cytoplasm that scarcely protrudes from the surface. The inner group of chromosomes becomes vesiculated, and a second polar spindle forms, on which the resolved chromosomes now collect. Here they divide, presumably typically, and

[8] MacBride ('96) states that in Asterina gibbosa parthenogenesis is normal. Greef ('01) found in one instance that the eggs of Asterias rubens developed without sperm. Hertwig ('90) found a few unfertilized eggs of Asterias glacialis and Astropectus segmenting. Delage has found occasional cleavages in unfertilized eggs of A. glacialis. Newman ('21) records similar results with Patiria miniata.

the daughter halves move to the poles (anaphase). Both sets soon become vesiculated, the vesicles unite into larger vesicles, and finally, in typical cases, two nuclei develop within the egg cytoplasm. They unite and sink deeper into the egg, where they become the segmentation nucleus. Thus the second polar body is suppressed, as Delage supposed. Its nucleus unites with the egg nucleus to form the segmentation nucleus.

Each blastomere gets 36 chromosomes i.e., the whole number which is the number also found in the cells of the blastulae. It is evident, then, that the parthenogenetic larvae of the starfish, after CO_2 treatment, contain the full complement of chromosomes. In this respect the larvae differ from those of the sea-urchin that are haploid.

Tennent and Hogue ('06) have also studied the effect of CO_2 treatment. Their results are somewhat different from those of Buchner. The difference may be due to different species being used in the two cases, or to a difference in the time of sojourn in the CO_2, or to the condition of the eggs at the time of treatment.

In the species used by Tennent and Hogue, Asterias forbesii and A. vulgaris, a shorter treatment with CO_2 was found advantageous. The eggs were placed in the CO_2 solution after the extrusion of the first polar body and left there for four minutes, then transferred to sea water. Eggs were preserved at five and then at ten minute intervals. After the extrusion of the first polar body 18 chromosomes were left in the egg. A second spindle forms, and a second polar body is formed, although it cannot always be seen on the surface since it is generally flattened against the egg. The 18 chromosomes left in the egg become the egg nucleus that moves toward the center of the egg. Two centrosomes appear just outside the nucleus on its polar side and move to opposite sides. Rays centering on centrosomes radiate into the protoplasm. The nuclear wall breaks down, the chromosomes move on to the equator of the spindle and divide, and the daughter chromosomes move to opposite poles. The egg then divides into two. The early cleavages that follow are normal.

It was with the greatest difficulty that the number of chromosomes of the dividing egg was determined, owing to their irregular shape, and distribution, their tendency to stick together and overlap. The final conclusion ('06) is that the haploid num-

ber is present. The difficulty in this case is due more especially to the occurrence of two kinds of individuals in one or in both species studied. Individuals with 9 and others with 18 chromosomes (reduced numbers) were met with in the course of their work.

The time required for parthenogenetic reagents to activate the egg has been studied by R. S. Lillie ('14, '16). Starfish eggs were exposed for 6 to 10 minutes to butyric acid in sea water. When returned to sea water they cleaved more or less regularly and developed into freely swimming larvae, blastulae and gastrulae. As many as 80 to 90 per cent formed "larvae." If under-exposed, 2 to 5 minutes in the acid, typical membranes formed, but cleavage was delayed and development did not get as far as the "swimming stage." If treated a second time by the acid, after an interval in sea water, the eggs developed further. Exposures to higher temperatures (30–35° C.) gives similar results. For each temperature a minimal exposure induces membrane formation (2 minutes at 32°); longer exposure more complete activation (8 minutes at 32°), but if exposed still longer (12 minutes at 32°) the eggs fail to develop. The time of exposure to produce each of these effects decreases rapidly as the temperature rises, being approximately halved by a rise of 1° C. The result suggests that the reaction must proceed until a definite quantity of reaction-product is formed. The failure to develop further by a short exposure can be made good by a second treatment that carries further the changes initiated.

Some light is thrown on the reaction by other experiments. When unfertilized eggs are put into sea water at 31° the optimum exposure is about 30 minutes; at 32° the same stage of activation is reached in 7 to 8 minutes; at 34° in 3 minutes; and at 36° in 1 minute. "The activation thus exhibits a high temperature coefficient ($Q_{10} = 200$ to 400); this coefficient is similar in its order to that of the decrease of viscosity or degelation of colloidal systems of gelatin in water under the influence of rising temperature." Lillie suggests that the viscosity may permit the combination of constituents which in the early stages are kept apart through their inability to diffuse. It is, therefore, the rate of diffusion (the reciprocal of viscosity) that determines the result; the specific nature of the chemical combination involved is not indicated by these experiments alone. Since bases and

acids differ entirely in their relation to the accelerating process in starfish eggs, and since butyric acid and ammonia are lipoid-soluble compounds and penetrate the egg readily, it is to be inferred that the contrast in their physiological effect is determined by their chemical properties, and not by their physical action on the colloids.

On the other hand, since in the sea-urchin egg all acids are alike in activating power, the rate of penetration is the chief factor in determining the reaction. Loeb and Hagedoorn ('13) had shown that the least effective duration of exposure to butyric acid solutions is halved by raising the temperature from 10° to 20°C., indicating also that the acid acts by chemical combination. If this were so the minimal time of exposure should also, according to the mass action law, be halved, but this does not appear to hold for the sea-urchin egg. Since bases as well as acids cause the membrane formation in the sea-urchin's egg, the membrane-forming effect appears to have no direct relation to the chemical nature of the agent employed.

R. S. Lillie has carried out experiments, extending over several years, on the egg of the starfish, to determine the nature of the change in the egg that is induced by those agents that bring about artificial parthenogenesis. Lillie was impressed, he says, from the beginning with the "various analogies" between the problem of fertilization and that of excitation of a nerve impulse. Some of the analogies may be, he thinks, superficial, but others possess certain fundamental physiological features in common, for both give a specific response to a change of condition that need not be specific. He is aware of course of one striking difference. When a nerve is stimulated the response takes place at once and is not rhythmical, while when the division of the egg is started by extraneous agents a rhythmic series of divisions takes place. Whether this difference is fundamental or whether it can be covered by considering the entire development as one process is perhaps open to discussion. There may be, in fact, two quite distinct problems here, one of which is concerned with the nature of the initial stimulus and its immediate cause, the other with the rhythmic nature of cleavage.

Lillie's work has brought to light a number of significant facts that have a bearing on the nature of the initial changes in the egg under artificial treatment. In his 1913 paper he pointed

out that, in the excitation of a nerve, the critical event is a temporary change in electrical polarization, and since the first observable change in fertilization (both by sperm and by artificial means) involves an initial increase in the permeability of the surface of the membrane ("its plasma membrane") this change should be accompanied by an electrical polarization of the surface membrane. Miss Hyde's work ('04) on Fundulus has supplied evidence that such a change does take place. The initial depolarization resulting from an increase in permeability that lasts about fifteen minutes is followed by a return to the original polarization.

It is known that anesthetics check the permeability of cells. Lillie utilized this relation in order to study the nature of the initial cleavage that takes place in artificial fertilization. Unfertilized eggs of the starfish were exposed for a short time to pure isotonic NaCl solution. One to five per cent may become "larvae." If, however, the eggs treated by NaCl are placed for 30 minutes either in a hypertonic sea water, or in a weak cyanide solution, more larvae develop. The same effect of after treatment can be obtained with ethyl ether, ethel urethane, chloral hydrate, chloretone, and various alcohols. Arbacia eggs do not respond to this treatment. Lillie's general conclusion is as follows:

"The view is put forward that the essential physiological effects produced by the two successive treatments in artificial parthenogenesis are opposite in character and correspond respectively to the depolarizing and the repolarizing phases of the stimulation-process in irritable tissues in general. The primary or membrane-forming treatment has a permeability-increasing and hence depolarizing effect upon the plasma membrane. This initial depolarization is probably the critical or determinative event in fertilization, as well as in stimulation. A return of the plasma membrane to or toward the original or semi-permeable and electrically polarized condition within a certain time (ca. fifteen minutes at 20 degrees), is, however, essential if normal development is to follow, otherwise cytolysis results. This recovery of the normal semi-permeability of the membrane, with the correlative electrical polarization, is favored by after-treatment with agencies (cold, cyanide, anesthetics, hypertonic seawater) whose general action is permeability-decreasing or anticytolytic. Hence, such after-treatment increases the proportion

of eggs that regain their normal properties and continue development."

In the following year ('14) Lillie studied the antagonism between salts and anesthetics. He had shown in earlier papers that the initiation of cleavage by pure isotonic solution of sodium or potassium salts may be prevented by calcium or magnesium chloride ('11). The indications are that the pure salt increases permeability; the antagonistic salt counteracts this permeability. Since anesthetics, as well as calcium and magnesium, make the plasma membrane more resistant to the permeability-increasing action of salt solutions, they ought also to prevent the cleavage-initiating action of these solutions. This was found to be true for the Arbacia egg. The addition of anesthetics to the pure salt solutions (KCNS and NaI) decreased the cleavage-initiating action of the salts. Great differences were, however, shown by different anesthetics. Chloral hydrate was less effective than alcohol for instance. How the effect is produced is uncertain. Lillie sums up his conclusions as follows:

"We reach thus the general result that the formation of fertilization membranes and the initiation of cleavage may be prevented by anesthetics when the parthenogenetic agent is a neutral salt, but not when it is a fatty acid. This contrast is what would be expected on the assumption that the essential action of the anesthetic is superficial, and consists in rendering the plasma-membrane more resistant to alterations of permeability. Hence the salt, which does not readily penetrate the unaltered egg and produces its effect by increasing the permeability of the plasma-membrane, is rendered less effective when the membrane has been rendered relatively resistant or stabilized by the anesthetic. The fatty acid, on the other hand, which penetrates the plasma-membrane readily under all conditions, by virtue of its lipoid-solubility, is not prevented in its action by anesthetics."

The effects of sensitizing unfertilized eggs of Arbacia by isotonic salt solutions, have been further studied by Lillie and Baskervill ('21). They have shown that an increase in susceptibility to subsequent treatment with hypertonic solutions may occur even when no visible change in the membrane is apparent.[9]

[9] The eggs were exposed to isotonic solutions of NaCl for 5 to 10 minutes at 20–22 degrees C, and treated subsequently for 20 to 45 minutes to hypertonic sea water.

In fact, the eggs may respond normally to sperm fertilization. The sensitizing effect of the salt may be decreased by the addition of $CaCl_2$ to the pure NaCl solution. Even a pure $CaCl_2$ solution may produce a sensitizing effect. The sensitized egg does not form a fertilization membrane, and the eggs may remain alive subsequently for 24 hours or more. The sensitized condition is not lost or reversed in sea water, but may persist as long as 48 hours. It is important to note that the results show that the physiological effect produced by the isotonic salt solutions, and by the hypertonic sea water, are different and cannot be reversed. Eggs exposed first to a hypertonic solution, and then to pure isotonic NaCl solution do not respond, in fact the second exposure acts injuriously on the egg.

It was also found that a brief exposure of unfertilized eggs of Arbacia to ultra-violet rays affects the eggs in such a way that they become more responsive to subsequent treatment with hypertonic sea water. Similar effects are produced by shaking the eggs for several seconds or minutes. Longer shaking injures the eggs. Lillie and Cattell ('25) obtained partial activation of the starfish egg by strong electric currents (that deformed the eggs), but not by moderate currents.[10]

Artificial Parthenogenesis of the Frog's Egg

Despite many attempts by different writers to bring about the development of the unfertilized frog's egg by treatment with different solutions, little more than a few imperfect cleavage furrows have been produced. But by means of a very different procedure Bataillon ('10) discovered a method that has given moderately successful results.

Three years before Bataillon published his results, Guyer ('07) had described an experiment with the frog's egg in which the development of unfertilized eggs was brought about by puncturing the egg with a capillary tube charged with lymph and blood. He observed no superficial cleavages and this, no doubt, led him to state that some sort of internal nuclear arrangement took place, which, he suggested, was due to a migration of the

[10] It is to be noted that the eggs were in silk bags in a strong current of cool sea water to keep down the temperature. The eggs were greatly deformed, presumably not by the current of water, but by the electric current.

injected leucocytes from the interior to the surface of the egg where these finally formed into one or two layers, each nucleus apparently acquiring a zone of protoplasm about it that led to cellulation. This injection method is in some respects the same as that employed later by Bataillon, and, in fact, the final conclusion arrived at by the latter has at least certain points in common with Guyer's. The very fragmentary nature of Guyer's work, and especially his conclusion that the development was possibly due to the substitution of a nucleus of a blood corpuscle for that of the egg, probably explains why it received little attention. On the other hand, the extensive series of experiments and observations made by Bataillon brought the necessary evidence showing that a new method of artificial parthenogenesis had been found. His experiments were soon repeated and the results confirmed by several embryologists (Henneguy, Dehorne, Brachet, McClendon, Herlant, Loeb and Bancroft), but as yet little or no improvement of the method has been made, although, at best only a minute percentage of eggs that have been punctured develop even as far as the larval stages.

Bataillon was led to his discovery by his observations on the eggs of the toad, Pelobates, inseminated with the sperm of Triton. The sperm punctures the surface, but does not take any further part in the changes that follow. The second polar body is given off and the cleavages begin after some hours. The results suggested the possibility that if eggs were punctured with a fine needle of glass or of platinum their development might be started. The partial success that followed this procedure led Bataillon to make further trials between the years 1911-'14. He reached the conclusion that puncturing alone is insufficient to induce division of the egg, and that an additional factor is necessary to carry the egg farther into the embryonic stages. This second factor is the introduction into the opening of some element of the blood. This second requirement Bataillon compared to Loeb's second or corrective agent that prevents the further cytolysis induced by the puncture of the surface. The true nature of this second element was never clearly made out by Bataillon, but is referred to as "matériel nucleaire étranger." The uncertainty of the results in individual cases is ascribed to the variability of the amount of the introduced, unknown material possibly of "nuclear" origin. Despite this uncertainty the essen-

tial point remained, namely, that only when some element of the blood is present is the result successful.

Later Herlant ('12, '13) undertook to study in detail the changes that take place in the punctured frog's egg; first, after puncture alone without the possible participation of elements from the blood, and, second, when the latter was present at the time of puncture. It is necessary in order to obtain eggs free from blood or lymph to squeeze them out of the cloacal opening, for if the eggs are obtained by the usual method of opening the abdomen and uterus, it is not always possible to eliminate the presence of blood or lymph.

When the egg (Rana fusca), *free from lymph,* is punctured with a glass needle the following changes take place. All the eggs respond by setting free a fluid which accumulates between the egg and its vitelline membrane (fertilization membrane) which is lifted from the surface. The eggs are set free and can rotate in response to gravity. The black hemisphere then turns upward. All the eggs give off the second polar body and the egg-nucleus is then formed (Fig. 245*a*). It migrates toward the center of the egg. After 40 minutes faint rays appear in the protoplasm centering near the nucleus. These slowly become more and more pronounced until the interior of the egg is occupied by a dense ring, pierced by rays (Fig. 245*b*). The nucleus lies at or near the center of the ring (75 minutes).

After two and a quarter hours the egg appears as in Fig. 245*c*. The rays in the condensed ring (shell) begin to disappear. After two and a half hours more, the nuclear wall disappears and the condensed chromatin becomes surrounded by a new delicate system of rays centering on one or two foci. Two centers appear at this time; the poles of a mitotic figure, that come into connection with the chromatin mass (the intermediate stages were not obtained). The poles of the spindle separate, and near each is found a reconstituted nucleus and small aster. A few creases appear later on the surface, but the egg never divides into two blastomeres. Still later four centers may develop in the egg, each with a well-defined mitotic figure (5 hours). In later stages a few cells may be formed resulting probably from the purely superficial position of the small mitotic spindles derived from the earlier nuclei. After 15 or 20 hours all eggs are dead.

Eggs that receive the double treatment i.e., eggs punctured

in blood serum, have in part the same history, but a new element is introduced that leads to a more normal cleavage and activation. Such eggs (that have received the double treatment) may show after 1½ hours a few small cytasters at the inner end of the path of the needle. No evidence of any introduced foreign body (leucocytes or clot) can be detected, but would scarcely be seen if present in the débris around the path of the needle. An egg preserved 1¾ hours after treatment shows the cytasters better developed. The egg-nucleus is also seen surrounded by a ring

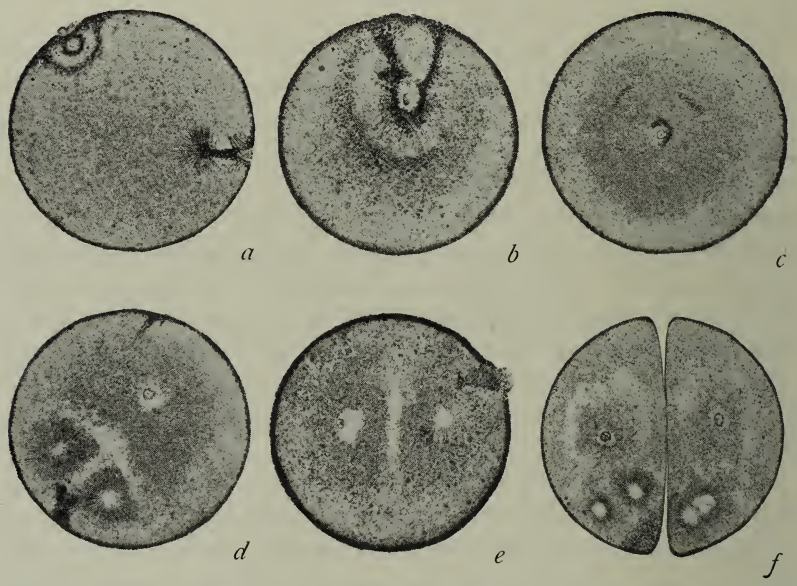


Fig. 245.—*a*, *b*, *c*, sections of frog's egg incited to develop by pricking with clean needle, showing the development of the aster near the path of puncture; *d*, *e*, *f*, sections of eggs pricked with needle in presence of blood (double treatment). (After Herlant.)

with its rays. A later stage (2¼ hours) contains much larger asters. The monaster around the nucleus is fading out. Another egg of the same age shows two much enlarged cytasters. There are present in this egg also a number of smaller asters in the region around the puncture. The monaster is still further faded out. Another egg of the same period (Fig. 245*d*) shows two large cytasters, that are encroaching on the disappearing monaster which appears as though it were being pushed to one side or rather invaded by the cytasters.

The following stages are incompletely described by Herlant, and it is not certain that he has put in order the series of events that leads to the most complete development of the egg. As the cytasters enlarge, according to his account, they encroach on the large central egg-aster. Meanwhile within the central aster a division figure has appeared (as in eggs with a single treatment), and, later, two daughter-nuclei are found in the egg widely separated. Herlant believes that the accessory asters do not bring about the division of the nucleus, but, as stated above, its division is caused by its own division figure. Since the cytoplasm of the egg after the single treatment does not divide, while after the double treatment the egg does divide, Herlant argues that the division is made possible, or brought about indirectly, by the cytasters—perhaps by coinciding in position with the two daughter-nuclei in the center of the degenerating, central aster.

It is obvious, however, that this machinery of division may often or even generally, fail to follow the simple scheme indicated in these selected cases. If more than two asters develop and influence the first division, the egg may divide into three or more parts, and the chromosomes may be irregularly distributed to the poles, or one pole may even fail to receive any of the chromosomes. The cell formed about the latter center might consequently act injuriously on the subsequent development. Such cases have been observed by Herlant. Even when the first division is into equal parts, other accessory asters may be present in one or both blastomeres and disturb their later divisions. It is also possible that one aster may outstrip the rest, hence the egg may fail to divide at all, or only irregularly later, etc.

With all these chances for irregularities in the early cleavage, we can understand why so few eggs cleave with sufficient regularity to lead to normal development. In fact this method of traumatic fertilization appears, from Herlant's evidence, far less perfect on the whole, than the improved methods of chemical fertilization that Loeb and others have worked out.

Most of the eggs of the frog that have received the double treatment fail to divide, and, amongst those that do divide, only a very small percentage show a regular cleavage. Amongst these again only a small number pass beyond the later segmentation stages. Rarely one gastrulates and forms an embryo. Even these embryos are as a rule abnormal, as Herlant observed.

Loeb succeeded prior to 1919 in raising twenty-one parthenogenetic frogs through metamorphosis. Of these, fifteen were males, three were females, and two were undetermined. In 1919, he carried sixty-five tadpoles to metamorphosis. Some of the material was turned over to Parmenter for examination. Of thirty-four tadpoles studied, twelve were males, two were transforming into males, eighteen were females and two were doubtful. Parmenter reports that both sexes have the diploid number of chromosomes. The exact number is not certain. In five clear cases, there were twenty-six, and in two cases twenty-seven chromosomes. Goldschmidt ('20) had found the diploid number in one of Loeb's metamorphosed frogs.

There has been much difference of opinion as to the chromosome number in parthenogenetic frogs. Bataillon ('04, '10, '11), reported the haploid number in young stages (seventeen hours old). Dehorne ('10), also found the haploid number in still younger stages. Brachet ('11), found the diploid number in an eighteen day tadpole. Levy ('13), found approximately the haploid number in swimming tadpoles, and later ('20) in abnormal tadpoles from eight to twenty-four chromosomes in the epithelium cells of the tail. Hovasse ('20, '22) examined a large series, more especially the younger stages. Of these, sixty-five were haploid, seventy-five were diploid, and fourteen had an aberrant number, and he also records varying numbers of chromosomes in the same individual. In this connection the results of Gunther and Paula Hertwig are to be taken into account (see below), where the change from haploid to diploid number of chromosomes has been definitely connected with the first division of the egg.

Since it has been shown by Brachet and Herlant that the second polar body is given off after pricking the eggs of the frog, a haploid nucleus is left in the egg. If this divides at the first division into two daughter nuclei, which separate, expectation is that a haploid embryo would result. If, on the other hand, the first nucleus should double its number of chromosomes before the first cytoplasmic division takes place, the embryonic cells would be diploid. Irregularities in the first or later divisions would account for the aberrant numbers reported by several observers. Other evidence makes it probable that the haploid embryos die before reaching the time of metamorphosis, and it is probable that most of the embryos with aberrant numbers of

chromosomes also die at an early stage. It may seem probable, therefore, that only the diploid embryos succeed in developing into frogs.[11]

Parthenogenesis in Annelids

Several methods of inducing artificial parthenogenesis in annelids have been found, and in one case at least (Lefevre '07) the success was remarkable. Mead's earlier experiments ('98) have already been mentioned. Loeb ('01) induced development in Chaetopterus with KCl and other salts as well as with dilute HCl, but as the "trochophore" stage was apparently reached without visible sign of cleavage, it is perhaps questionable whether this sort of development deserves to be described as artificial parthenogenesis. F. R. Lillie ('02, '06) described more fully such non-cellular embryos of Chaetopterus, which had developed from eggs fertilized in salt solutions that had suppressed or imperfect cleavages. Scott has obtained similar results with Amphitrite, and Scott and Fischer have made some further observations on Amphitrite. Bullot ('04) found in Ophelia that after treatment with KCl and NaCl only those eggs that segmented produced larvae. Scott ('06) obtained abnormal cleavage in Amphitrite after treatment with $Ca(NO_3)_2$ and also by mechanical agitation. Ciliated bodies were obtained that only remotely resembled embryos. Lefevre ('07) got only irregular cleavage after treatment with $MgCl_2$, $Ca(NO_3)_2$, KCl or NaCl, but excellent results by treating the eggs of Thalessema with dilute solutions of either strong or weak acids. His results may now be described in some detail, since they give further information concerning the internal changes of the egg.

The eggs of Thalassema, removed from the ovary, were placed in one or the other of the following solutions:

17 cc. M/10 HNO_3 plus 83 cc. sea water, for 5 minutes.
15 cc. M/10 HCl plus 85 cc. sea water, for 5 minutes.
10 cc. M/20 H_2SO_4 plus 90 cc. sea water for 8 minutes.
12 cc. M/20 Oxalic acid plus 88 cc. sea water for 8 minutes.
15 cc. M/10 Acetic acid plus 85 cc. sea water for 5 minutes.

[11] Witschi reports that twenty-six chromosomes (haploid thirteen) are present in Rana temporaria. In the male there is an XY pair.

Great variability in the results was observed, even with the same solution when different individuals were used. Also the length of time of immersion in the acid had a very marked effect on the number of embryos produced. Graded series of each solution were usually employed and the ones that gave the best results were followed. For example, in one experiment 60 per cent of the eggs developed into active swimming embryos indistinguishable from normal embryos. The eggs had been left 5 minutes in 15 cc. M/10 HCl plus 85 cc. sea water. In another lot of eggs, from the same female left for six minutes in the solution, only 5 per cent developed at all, and none went beyond the early cleavage stages.

While in the acid, no membrane is formed, but soon after the transfer to sea water a fertilization membrane appears. It may be recalled here that the ovarian nucleus is still present in the egg when obtained. It normally remains intact until the sperm enters, and after its wall dissolves, the polar bodies are extruded. The same change in the nucleus takes place after treatment with the acid solution, but the eggs do not round out as quickly as they do when the sperm enters. The egg throws off the membrane (or the membrane is lifted from the egg) leaving fine strands connecting the surface of the egg with the membrane. This occurs also in the normally fertilized egg.

The nucleus moves from the center into an excentric position (Fig. 246*a*). Consequently in the parthenogenetic egg the first polar spindle lies nearer the surface than in the fertilized egg in which the nuclear migration does not occur. Normally the first polar body forms in 20 minutes, but only after 45 to 90 minutes after artificial activation. A second polar body forms immediately under the first. In the normal egg the first polar body after extrusion divides once, but the second does not divide; in the parthenogenetic egg both polar bodies divide several times and form a little heap of cells outside the egg above the pole.

The first cleavage may not appear until 2 to 3½ hours after treatment. "The early cleavages are closely similar to the normal in a great many cases and in favorable experiments where the optimum conditions were present, the segmenting eggs could not be distinguished from the controls fertilized with sperm, except in the lack of uniformity in the rate of division exhibited by the former, especially during later stages." The first cleavage divides

the egg into equal parts. The second cleavage is again equal. The third cleavage forms eight cells, the upper (polar) four constituting the first quartette of micromeres. The second quartette then forms from the macromeres (Fig. 246*c-f*). An unequal division of the first quartette produces the trochoblasts. In all these respects the induced cleavage is exactly like the normal cleavage. A normal gastrulation takes place and a per-

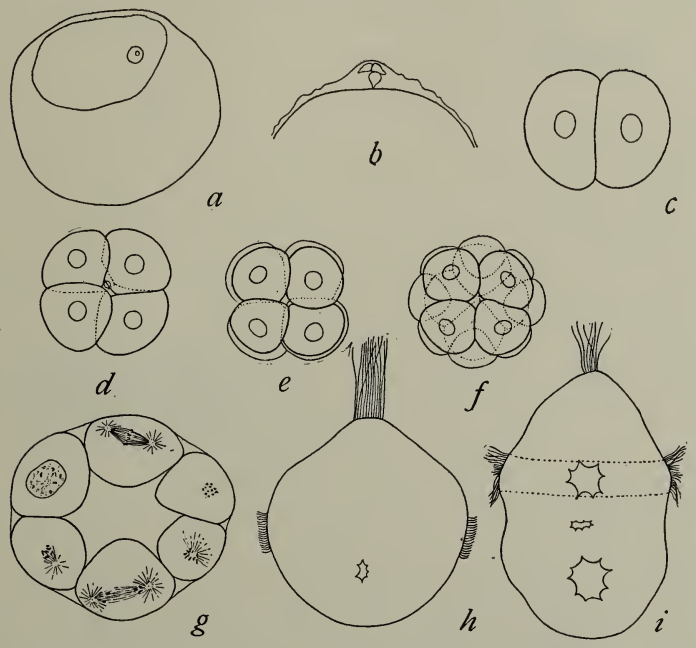


FIG. 246.—Cleavage of eggs of the annelid, Thalassema, artificially fertilized. (After Lefevre.)

fectly formed trochophore develops in the most successful cases (Fig. 246*h-i*).

Sections of the treated eggs show two asters just outside of the nuclear wall (15 minutes after treatment). No secondary asters were seen although such have been recorded by Griffen ('99) for the normal egg. A spindle forms and the reduced number of chromosomes appears. The chromosomes divide and go to the poles of the first polar spindle. Before the close of the first mitosis two centrosomes present at the end of the inner spindle begin to separate to form the spindle for the second polar

body. The second polar body is next given off. The egg-nucleus now moves to the center of the egg and a resting period of one to two hours ensues.

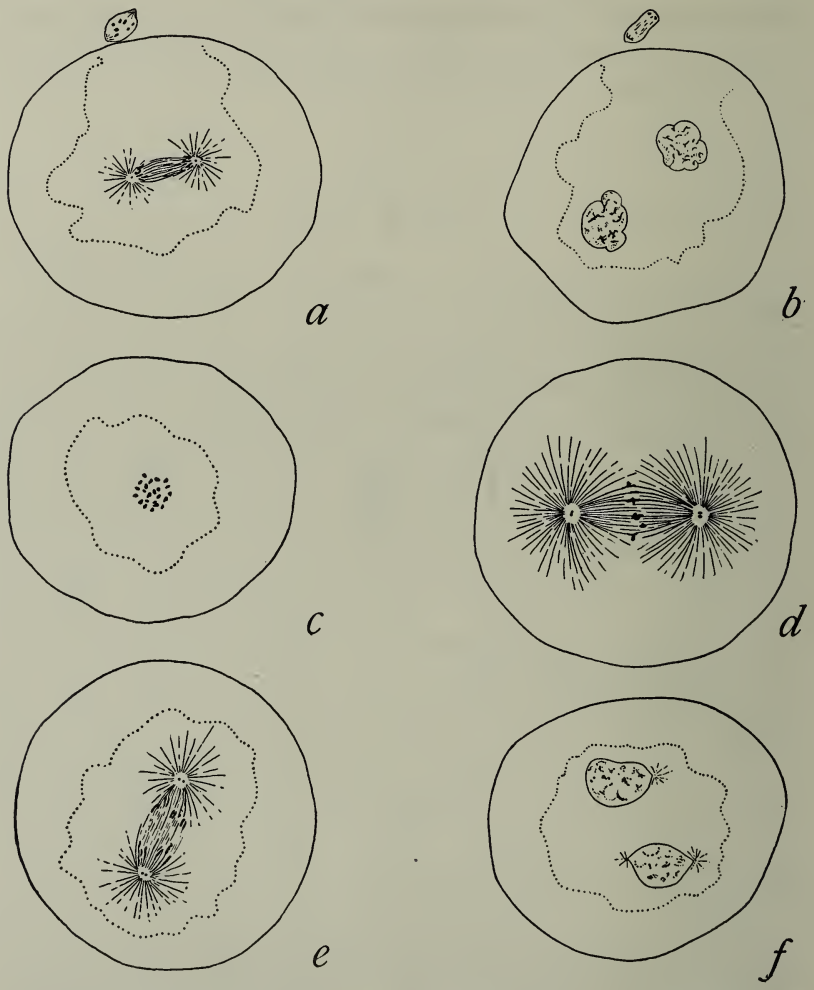


Fig. 247.—Sections of eggs of Thalassema, artificially fertilized. (After Lefevre.)

The first indication of the preparation for cleavage is seen in the simultaneous appearance of two minute asters on opposite sides of the nucleus. A spindle forms with the haploid number of chromosomes at its equator and a normal division figure results

(Fig. 247*d* and *e*). Even before the egg has divided the centrosome in each blastomere divides and its halves begin to separate, but it is not possible to trace them through the resting stage that follows. In later cleavage stages regular mitotic figures develop.

The most interesting effects of the activation are seen in cases, when, after treatment with the acid, the second polar body is not given off. Such eggs segment, however, and may produce larvae indistinguishable from those from eggs that have thrown off both polar bodies. Such cases are not associated with special solutions or treatments, but may appear at any time even with the optimum solutions. In eggs that extrude only the first polar body, the second spindle fails to assume the usual radial position but sinks down into the egg. Here it completes its division and produces two resting nuclei. It seems probable that these two nuclei come together and a new mitotic figure develops with the combined nuclei in its middle. The diploid number of chromosomes is then expected. While the number of chromosomes was not definitely ascertained, it was at least seen in eggs presumably having these antecedents, that the number is greater than the haploid number. Embryos from such eggs would then be expected to have the whole number of chromosomes.

Eggs that fail to extrude even the first polar body were also found to develop into swimming larvae. The percentage of developing eggs was as high as when both polar bodies were extruded. The first spindle remains near the center of the egg. The chromosomes are diads and not single rods. After division two resting nuclei are formed, and it seems probable that they reunite to form a cleavage nucleus. In some cases it seems probable that the two first-formed nuclei do not unite, but each divides again to form four nuclei. Whether they fuse could not be determined, but this is probable because in later stages of the same series a single cleavage nucleus is formed, there being no extraneous nuclei. The number of chromosomes is more than 12, but the exact number could not be determined. If in these two divisions the chromosomes behave as they do in the two polar divisions the number expected would be four times twelve or forty-eight (tetraploid).

In a number of experiments in which no polar bodies were extruded, the first maturation spindle, which is very large, extends

across the egg with tetrads at its equator. Since many eggs in these series become divided into two cells, it seems very probable that the first maturation spindle has been changed into the first cleavage spindle. Whether normal development follows is not stated.

Fischer ('02) has brought about the development of the unfertilized eggs of Nereis kept in a solution of sea water plus KCl. These eggs placed for 45 minutes in 80 cc. sea water plus 20 cc. KCl 2½ M. gave segmentation stages and later swimming larvae. Some of the eggs that first segmented divided into two, but many eggs divided into more than two parts. The figures show a very irregular cleavage, and the swimming embryos described as "trochophores" are no doubt monstrous forms like those obtained from annelid eggs by other observers. Segmentation stages and swimming larvae were also obtained by placing eggs in 30 cc. cane sugar 2 M. plus 70 cc. sea water. The results are due, Fischer thinks, to the osmotic pressure of the solutions and not to the presence of electrolytes in the KCl mixture.

F. R. Lillie ('02, '06) studied the effect of solutions of CaCl on the unfertilized (and fertilized) eggs of another annelid Chaetopterus. The treatment causes the same movements of the cytoplasmic materials seen after fertilization. This is especially marked in the movements of the ectoplasm. Many of these eggs fail to divide, but the nucleus passes through several successive stages of activity with the result that a large number of chromosomes are produced which become inclosed in a large central nucleus. Cilia later develop over one hemisphere, or even over the whole surface, which cause these monstrous forms to move about. The redistribution of the yolk and other materials in the eggs, to nearly the same locations that they occupy in the normal embryo, gives these monsters some resemblance to normal trochophores; but there is not much else about them to suggest the sort of differentiation seen in normal embryos. It may appear that some differentiation (such as the development of cilia) may take place without cell division, but it takes a considerable stretch of the imagination to identify these monstrosities with normal embryos.

Allyn ('12) has shown that the unfertilized eggs of Chaetopterus may be incited by many different reagents to cleave irregularly, and sometimes to produce "swimmers" that are not de-

scribed. The best results were obtained by heat (32.5° to 34.5°) for about 40 minutes.

The development of the unfertilized eggs of another annelid, Amphitrite, when treated with several salt solutions (KCl, KNO_3, $CaCl_2$, etc.) has been described by Scott ('06). The results, in general, are the same as those described by Lillie, but irregular cleavages, or partial cleavages in the early stages with fusion of cells in later stages, are more frequently described. Ciliated monsters develop whose only points of resemblance to normal embryos are the ciliated areas such as appear in normal embryos and the presence of brownish diffused pigment. Without cellulation no normal development occurs, according to Scott.[12]

The unfertilized eggs of Nereis do not respond favorably to the agents that arouse other eggs to parthenogenetic development. This is all the more surprising since other annelid eggs are readily excited to develop by salt solutions and by several other agents. Just ('15) has nevertheless succeeded in calling forth artificial fertilization in Nereis by means of heat. The female is dried on filter paper and cut open. The eggs are collected in a *dry* watch glass. They are then put into a small amount of sea water that has been heated to 34° or 35° C. In favorable cases every egg secretes jelly at once; the polar bodies are given off, and 90 to 100 per cent of the eggs will cleave. Not more than 20 per cent of "swimming embryos" come from these eggs. It is stated that some of these swimmers are trochophores closely resembling the normal ones. There is, however, no detailed account of the cleavage and not sufficient evidence as to the normal structure of these trochophores.

If the females are first put into the warm water, and there cut open, a small percentage gives off jelly and cleaves. The presence of body fluids or tissues may interfere with this reaction, but it is significant that, in a footnote, Just states that "with dry eggs one must be careful, for the mere drying will initiate cleavage as I have found. Eggs left on filter paper for from five to twenty

[12] Treadwell ('02) obtained ciliated masses from eggs of the annelid, Podarke, treated with KCl.

Fischer ('02) appears to have obtained abnormal ciliated bodies which he spoke of as trochophores, by treating the eggs of Amphitrite with $CaCl_2$ solutions. There can be little doubt that what he saw were these same monstrous forms described by Scott.

minutes form jelly, a small per cent cleave and a few swim." It may be that the preliminary drying predisposes the eggs toward parthenogenesis which this warm water still further accentuates.

If the eggs of Nereis are first collected in sea water and then placed in the warm water, a small percentage only gives off the jelly and cleaves but no normal embryos result. Even a very small amount of water suffices to interfere with successful parthenogenesis by heat. Just interprets this as due to partial loss of fertilizin, but it is quite possible that other changes take place that have nothing to do with the production of fertilizin. It is significant that washed eggs may be fertilized if the process is not carried on too long.[13]

General Considerations

The extensive literature of artificial parthenogenesis shows only too clearly how futile it is at present to speculate as to the chemical reaction that starts an egg on its course of development. Whether the effect of the artificial agent causes a change only at the surface as generally supposed, or whether the change only begins there, can not be positively asserted. While it is not very enlightening to speak of this effect as the removal of a block that holds the egg in check, such a view has the merit, at least, of throwing the emphasis back on the egg itself as the principal actor in the event, but unless the nature of the block can be defined the statement is only a figure of speech. The initiation of development has also been said to be due to a stimulus, but unless the nature of the stimulus can be defined, the comparison has little or no value. A change in surface tension has

[13] Kostanecki has studied the changes that take place when the unfertilized eggs of the mollusc Mactra are treated with salt solutions (NaCl, $CaCl_2$, and $MgCl_2$). When removed from the body the normal egg still contains the large ovarian nucleus, which remains intact until a sperm enters. It then gives off its two polar bodies in a normal fashion. The sperm brings in (or produces) an aster that forms the mitotic figure for the first division.

When unfertilized eggs are treated with salt solutions of the right concentration the nucleus breaks down and the polar spindle is formed. Favorable treatment leads to the extrusion of the two polar bodies and finally to cleavage. In solutions that are less favorable one or both of the polar bodies may fail to be given off and an imperfect division results. In other cases several asters may develop leading to irregular cleavages. In none of the cases were normal embryos obtained.

also been suggested, but how such a change could start development is as difficult to explain at present as the observations themselves. Loeb has laid much emphasis on the cytolysis of the surface layer, but the nature of the special kind of cytolysis and its chemical equivalent is left unexplained. It does not seem probable that the instantaneous effect of the penetration of the tip of the sperm could cause such an effect in normal fertilization, even granting that the influence starts at the penetration point and passes around (or through?) the egg. Until more is known of the chemico-physical changes that take place both in normal and in artificial fertilization, the suggestions that have been made cannot be considered more than speculative. The fact that unfertilized eggs may be induced to develop into normal embryos by artificial agents of the most diverse kind, rather than the hypotheses to account for the change, is the outstanding feature of all this work.

Combining Artificial with Natural Fertilization

Beginning in 1906 Herbst published a series of studies (ten in all) dealing for the most part with the eggs of sea-urchins that were first artificially stimulated towards parthenogenetic development, and then fertilized by sperm of another species of sea-urchin. When these hybrid plutei were compared with hybrid plutei from normal eggs it was found that they were more like the maternal type. A study of the changes that take place in these eggs before and after fertilization has shown why these plutei are more maternal than are ordinary hybrids.

The results of the two principal papers may be given in some detail: the first, dealing especially with the kinds of embryos obtained; and the second, with the changes that take place in the eggs as revealed by sections.

Herbst found ('06) that when unfertilized eggs of Sphaerechinus are placed for a short time in sea water to which a little acetic acid has been added (50 cc. sea water to 3 cc. $\frac{1}{10}$ N acetic acid), then transferred into sea water plus sodium hydroxide (100 cc. plus 1.5 cc. $\frac{1}{10}$ N NaOH), then back to sea water, where they are fertilized (after two and a half hours) with sperm of Strongylocentrotus, the resulting plutei are more like the maternal type of pluteus than like hybrid plutei from untreated eggs (Fig. 248). In the following year Herbst ('07) made further

experiments in order to determine how far the shove towards parthenogenesis is necessary in order to swing the balance to-

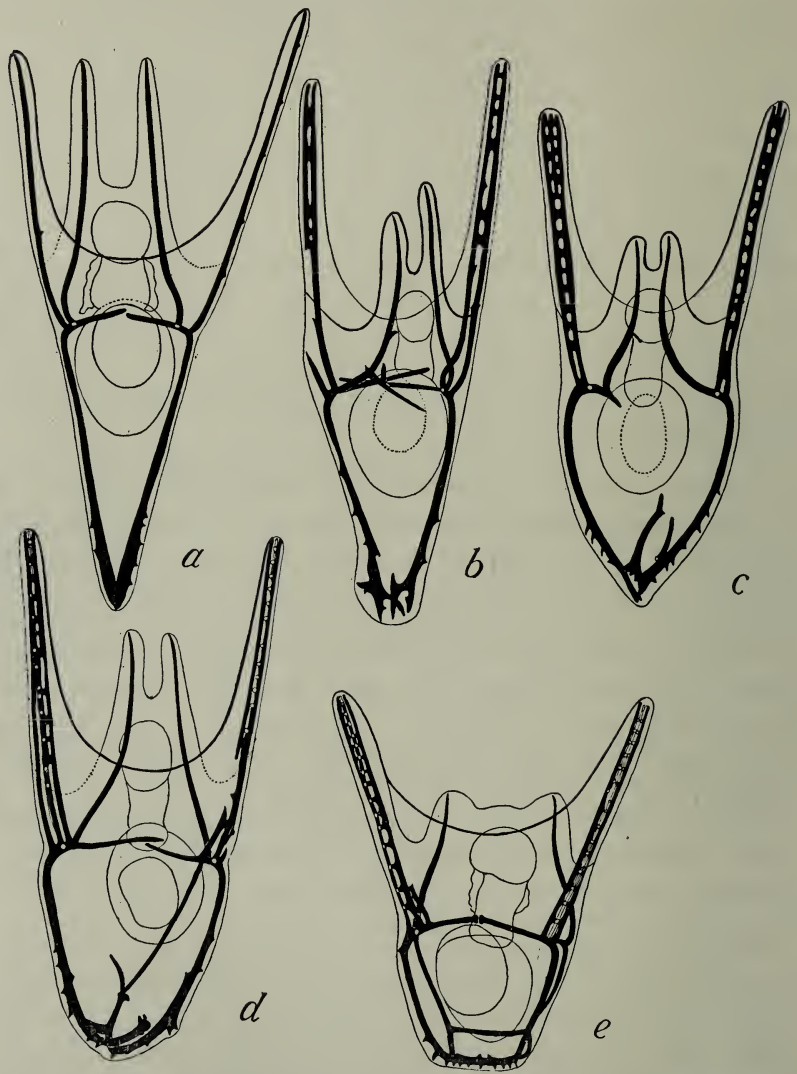


Fig. 248.—*a*, normal pluteus of Strongylocentrotus lividus; *b*, hybrid pluteus (Sphaerechinus ♀ by Strongylocentrotus ♂) from untreated eggs; *c*, and *d*, hybrids from treated eggs; *e*, parthenogenetic Sphaerechinus pluteus. (After Herbst.)

wards the maternal side. Since these results are more detailed than the former, and since other interesting kinds of plutei were

found, the later account may be followed. It may be stated in advance that the results are due, in cases where union of the egg and sperm-nucleus occurs, to the doubling of the egg's

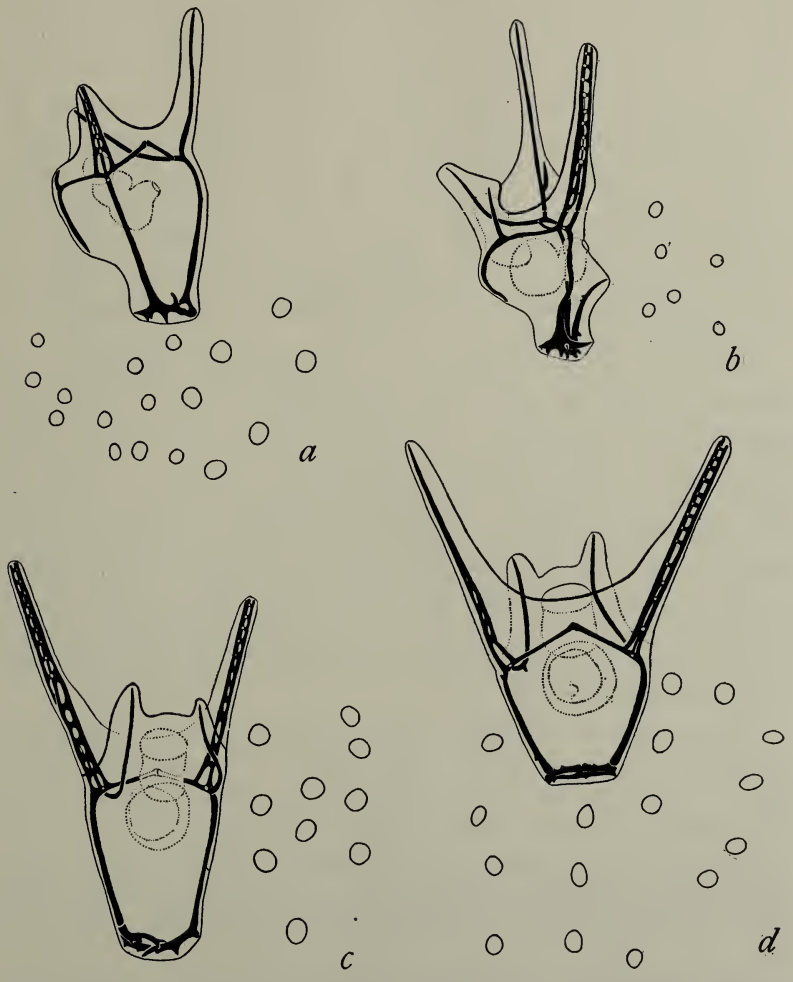


Fig. 249.—Plutei from eggs treated with parthenogenetic reagents, and subsequently fertilized, they are partly matroclinous, and partly patroclinous. (After Herbst.)

chromosomes as a result of their treatment with parthenogenetic agents. After fertilization such eggs are triploid. Two sets of chromosomes are contributed by the egg, and one set by the

sperm. Since, however, this union is often irregular, several kinds of mixed types are theoretically possible, and, in fact, occur.

Herbst made a study of the size of the nuclei, and found that in those plutei that show maternal characters the nuclei are larger than haploid nuclei, often twice as large or even larger (Figs. 249*c*, *d*). This result agrees with observations that before division a monaster stage appears, which means that a doubling of the maternal chromosomes takes place. When these nuclei are ready for division the diploid number of chromosomes is present. If a monaster appears twice before division the tetraploid number of chromosomes may be formed. In some of the hybrids with maternal characteristics, the nuclei are even larger than in the control composed of strictly parthenogenetic plutei. This difference could be explained if a sperm-nucleus had also combined with a diploid egg-nucleus.

Plutei are also present that are maternal on one side and partially hybrid on the other (Fig. 249*a*). The nuclei of the maternal side are smaller than those of the hybrid side (Fig. 249*a*). This condition may be explained as follows. After the egg-nucleus has formed a spindle, the sperm-nucleus units with one of its daughter halves. As a result the diploid side is expected to be strictly maternal, and the triploid side partially paternal (Figs. 249*a* and *b*). These are called partial thelykaryotic types. The nuclei on one side are larger than those on the other, and the skeleton is more maternal also on the side with the smaller nuclei.

In the same culture, plutei were found that had, throughout, small nuclei, as small as the smaller nuclei of the partially thelykaryotic larvae; some of these plutei are purely maternal in type (Fig. 250*a*) and each arises probably from the egg-nucleus alone, the sperm-nucleus having contributed no chromosomes. Other small-nucleated plutei are purely paternal in type (Fig. 250*c*). These probably arise from sperm-nuclei alone. A few larvae of this type may have regions of larger nuclei, and are, then, presumably mixed, i.e., to some extent partial thelykaryotic types (Fig. 250*b*).

In his later work (seventh study), Herbst gives a detailed account of a similar series of eggs, preserved at intervals, that had, as before, first received the parthenogenetic treatment, followed by fertilization with the sperm of another species. In many

respects these eggs are more uniform in the earlier stages than in the former cases and are, therefore, better suited to show, in a series of sections, what takes place; but since most of the embryos obtained were only of one type, and since no bilateral paternal-maternal embryos were obtained, this series probably

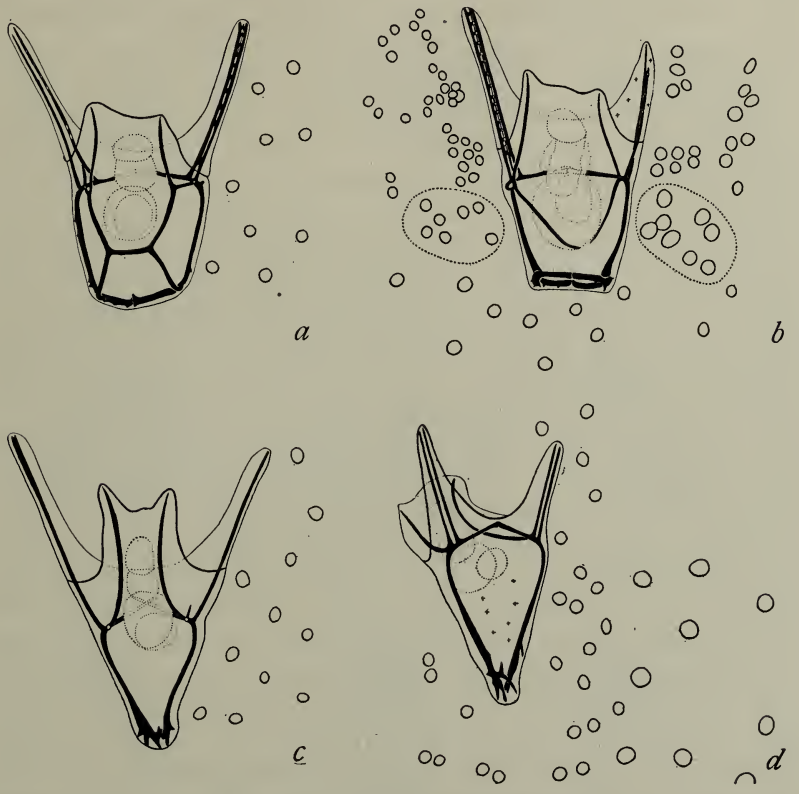


Fig. 250.—Plutei from eggs treated with parthenogenetic reagents; *a* purely maternal pluteus from egg-nucleus alone; *b*, asymmetrical partial thelykaryotic larva like Fig. 249, *a* and *b*; *c*, small nucleated pluteus of paternal type, probably from sperm-nucleus alone; *d*, partial arrhenokaryotic larva. (After Herbst.)

does not give as wide a range of possible combinations as the other series had given.

In this experiment the unfertilized eggs of Sphaerechinus were put into a mixture of sea water and iso-valerianic acid (200 cc. sea water plus 12 cc. $\frac{1}{10}$ N acid) for five minutes, then returned to sea water, and thirty minutes later fertilized with the sperm

of Strongylocentrotus. At the time of fertilization there is near the nucleus a clear zone of protoplasm with radiating fibres. Two hours later the eggs began to divide. Sets of these eggs were preserved at intervals, and later cut into sections.

The effect of the short sojourn in the iso-valerianic acid is to start parthenogenetic development. The nucleus becomes larger and its wall disappears. At the same time a clear protoplasmic zone appears at or around the nucleus from which rays extend out into the egg. This cytaster with a single center is called a monaster. The chromosomes, when set free from the nuclei, divide, which doubles their number. In the absence of a spindle, the daughter halves of the chromosomes do not move apart to opposite poles, but remain scattered at the edge of the monaster. Nuclear sap collects about them as they pass into a resting stage. While these changes have been taking place a spermatozoön has entered, and has begun to move toward the resting egg-nucleus. Sections of eggs taken at intervals make clear the successive changes. Ten minutes after insemination the egg has reached the stage shown in Fig. 251*a*. There is a large monaster with the chromosomes at one side. A sperm and its cytaster are seen near the periphery. Ten minutes later (Fig. 251*b*) the rays of the monaster are fading out and the chromosomes have become vesicular. Fifteen minutes later (Fig. 251*c*) the egg-nucleus has been reconstituted, and the monaster is disappearing, while the sperm and the sperm-aster have nearly reached the egg-nucleus. After another fifteen minutes (Fig. 251*d*) the sperm-nucleus has reached the egg-nucleus, and about its aster new rays are developing. In other eggs of this same set, the sperm-aster has divided and its poles are moving apart (Fig. 251*e*). Fifteen minutes later the chromosomes again appear in the egg-nucleus with which the sperm-nucleus is sometimes fused (Fig. 251*f*). In other eggs the sperm-nucleus may remain some distance from the egg-nucleus. From this time onward there is a great deal of variation found in the behavior of the sperm-nucleus. It is sometimes found on the spindle along with the chromosomes of the egg (Fig. 251g). In such cases the sperm-nucleus may remain condensed, in other cases it may show signs of being resolved into its constituent chromosomes, but these are generally stuck together in clumps, and rarely show typical forms (Figs. 251*h*, *i*). In other eggs the condensed sperm-nucleus may lie at or

near one pole of the spindle (Fig. 252*a*) or, as stated above, it may lie some distance from the spindle (Fig. 252*b*). When

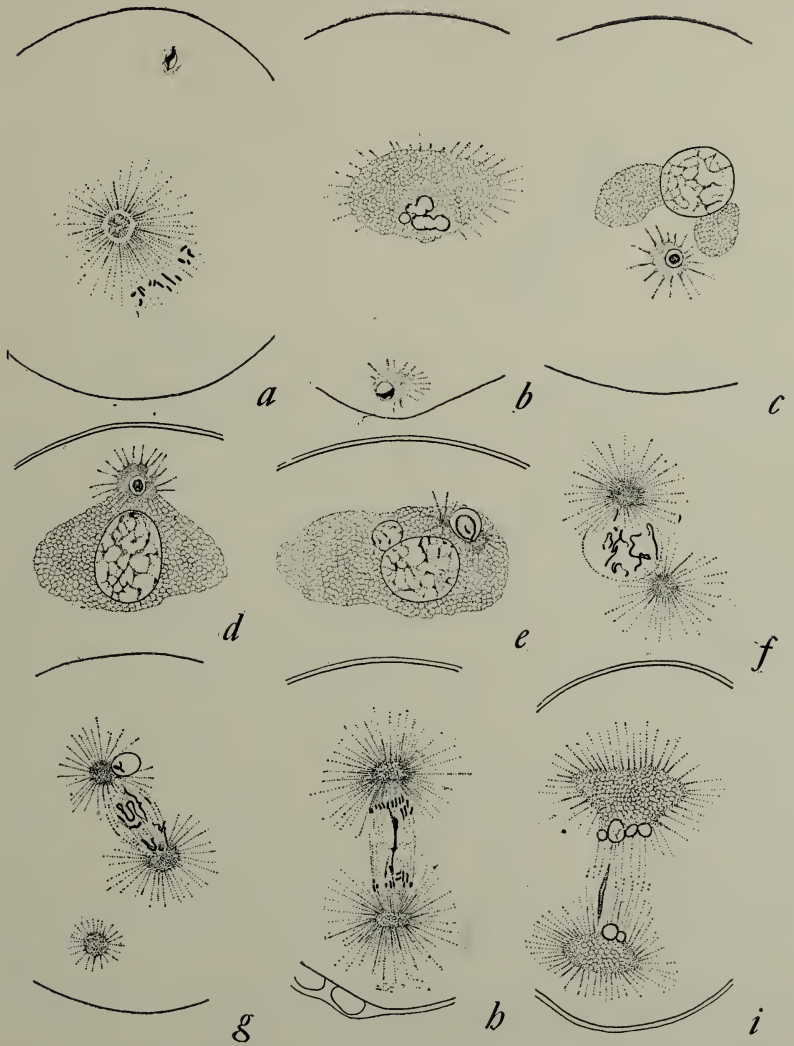


FIG. 251.—Eggs of Sphaerechinus that had been treated with valerianic acid solution, and later fertilized with sperm of Strongylocentrotus. For details see text. (After Herbst.)

the egg-chromosomes reach the poles of the spindle, and the two daughter nuclei are formed, the protoplasm divides into two

more or less equal-sized blastomeres; the sperm-chromosomes or sperm-nucleus may be present in a variety of conditions. When the sperm-chromosomes are present, some of them at least may

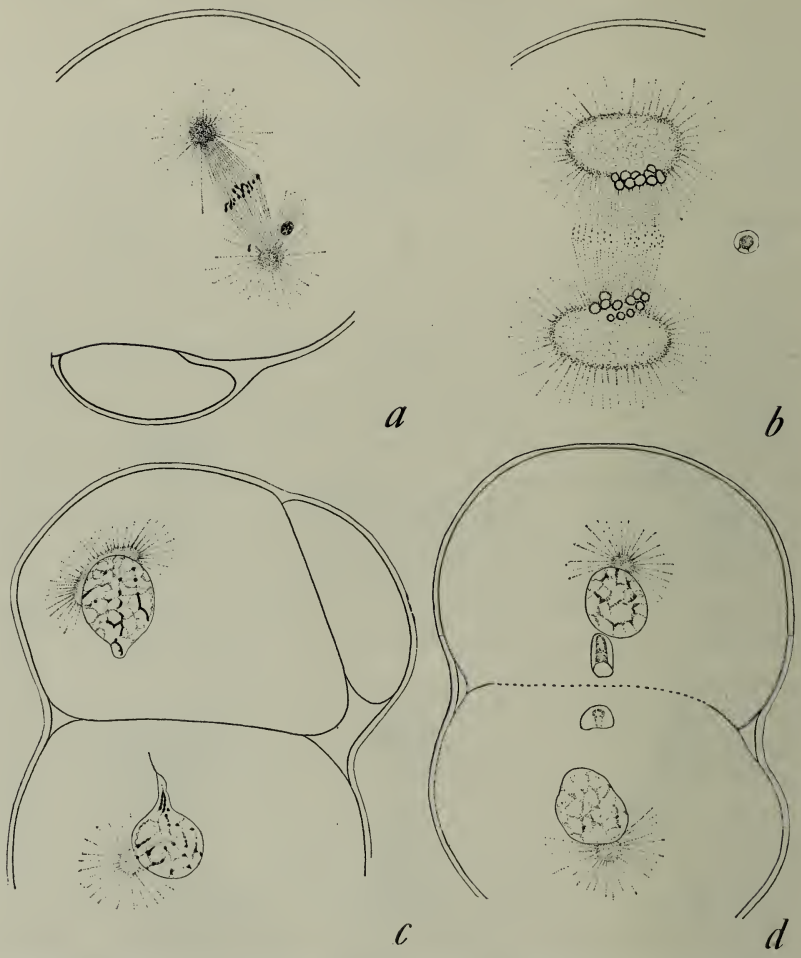


FIG. 252.—Eggs of Sphaerechinus as in Fig. 251. For details see text. (After Herbst.)

reach the poles (Fig. 252c) and join the egg-chromosomes, or the sperm chromatin may be left in the middle of the spindle (Fig. 252*d*). Frequently the whole condensed sperm-nucleus is carried over, possibly into one of the first two blastomeres.

These and other observations show that, in this series of eggs,

the sperm-chromosomes play a very secondary rôle in the subsequent development, although the asters for the first division appear to come in most cases from the sperm, or, at least, arise near the sperm-nucleus and under its influence. These observations on the eggs indicate that the main part of the chromatin is derived from the egg-nucleus which has doubled the number of its chromosomes as a result of the treatment with the acid. The eggs, therefore, appear to be in the main parthenogenetic (except, as stated above, for the sperm-aster) and it is not surprising, therefore, to find in this series that all the plutei are more nearly like the maternal type. In fact, it may be seriously asked whether the nucleus from the male has taken any part in the development further than to supply the sperm-aster.

From the variety of conditions met with in the first division it is to be expected that when the first two cells again enter upon division many differences will be met with at the next division and this, in fact, is the case. In Fig. 253*a*, an egg is seen in which the spindles for the next division are present. The one in the right hand cell seems normal; that in the left hand cell is less advanced, and within its nucleus the sperm-chromatin appears to be present in a less advanced stage than the egg-chromatin. In the same egg a small cell had budded off at the first division. It shows here the division products of another sperm-nucleus. In Fig. 253*b* the upper cell shows an undivided sperm-nucleus near the nucleus of the cell. In Fig. 253*c* remains of the sperm-nucleus are seen in both cells near one corner, and also parts, presumably of sperm-chromatin, inside the lower spindle. In Fig. 253*d*, traces of sperm-chromatin are seen near the equator of the spindle. In Fig. 253*e*, a condensed sperm-nucleus has fibres attached to it from one of the poles of one of the second spindles. In Fig. 253*f*, there is present in the right hand cell, which is in the last stage of mitosis, a condensed sperm-nucleus.

It is obvious that the sperm-chromatin lags behind in many cases, and sometimes is not even resolved into its constituent chromosomes. It is possible that as development proceeds some of the chromatin may be incorporated with the egg-nucleus and behave more normally, but it seems more probable that it will be further eliminated in later stages. It may even be questioned whether, when only a few of the sperm-chromosomes become in-

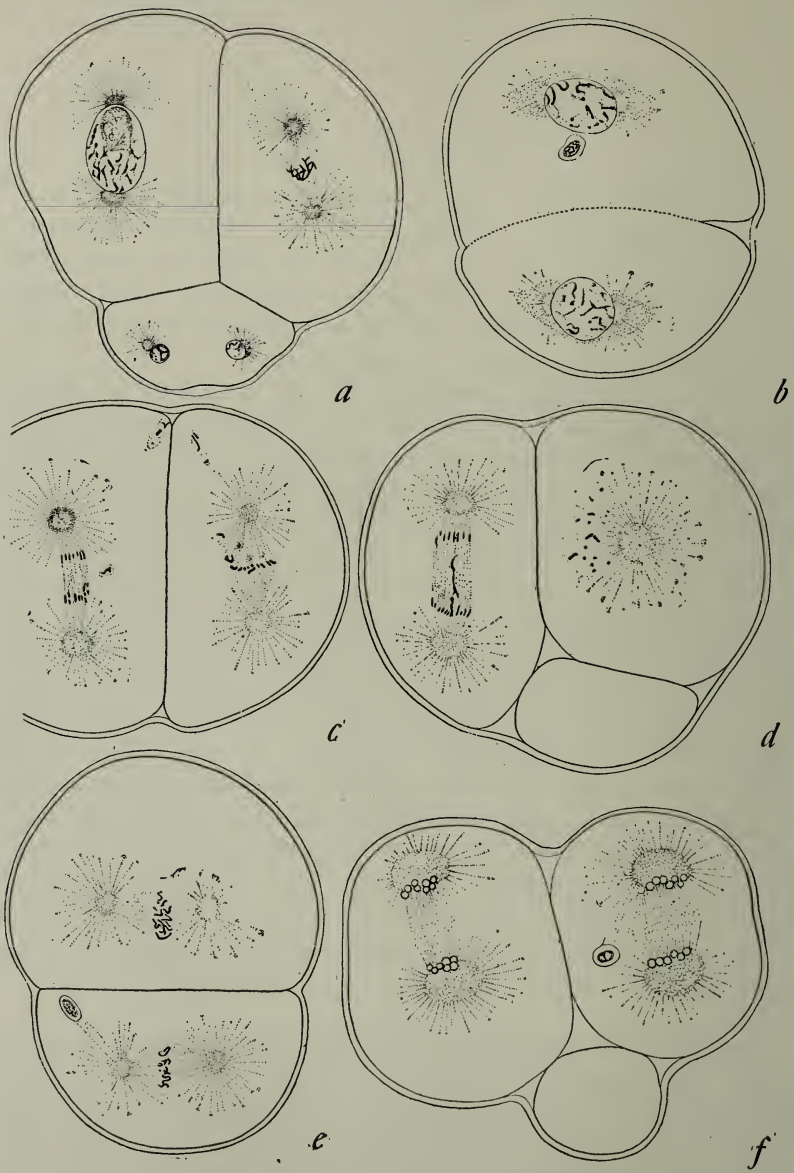


FIG. 253.—Eggs of Sphaerechinus as in Fig. 251. For details see text. (After Herbst.)

corporated with the egg-chromosomes, normal development can take place. Herbst is inclined to think this possible, but genetic evidence from other sources makes this view rather questionable.

Hinderer has studied ('14) unfertilized eggs of Sphaerechinus that had been first treated with CO_2 in order to start parthenogenesis and then subsequently fertilized by the sperm of Strongylocentrotus. He found a much greater regularity in the behavior of the male and female pronuclei than in Herbst's experiments, either because the parthenogenetic agent acted less strongly on the eggs, or because the eggs were fertilized at a different stage in their development. Correspondingly he found only two types of hybrid larvae, one with smaller nuclei whose plutei had a skeleton like that of the normal hybrid pluteus, the other with larger nuclei whose plutei had a skeleton more like that of the maternal type of pluteus.

The unfertilized eggs had been first put into a solution of 70 cc. CO_2 sea water + 30 cc. sea water, where they remained for five hours when the eggs were returned to sea water. Fifteen hours later those eggs that had not developed by parthenogenesis were fertilized with foreign sperm, and preserved at intervals. As a result of the CO_2 treatment the nucleus of the egg had doubled or trebled its size. It had undoubtedly passed through one or two monaster stages before the eggs were fertilized, during which the chromosomes had doubled once or twice. Sections of eggs preserved soon after fertilization showed that the sperm had entered these eggs and fused with the egg-nucleus. About an hour-and-a-half after fertilization the nuclear wall disappears and a spindle appears. The chromosomes become arranged on the spindle. Their number is difficult to determine accurately. It is known that the egg of Sphaerechinus contains 18 or 20, probably the latter number, while the sperm of Strongylocentrotus carries about 18 chromosomes. Hinderer found that when the eggs with the smaller sized nuclei divide there are present about 38 chromosomes, of which 20 presumably came from the egg-nucleus, 18 from the sperm. In such cases the normal diploid number is present and no doubling of the egg chromosomes has occurred. It is from these eggs that the typical hybrid with intermediate skeleton develops. In other cases a larger number of chromosomes were found, namely, 48, 46, 42, 50+. Here it is probable that the egg had doubled its chromosomes (20 + 20),

and to these the sperm had also contributed a certain number of chromosomes. Since no eggs were found with three times the haploid number of chromosomes it is probable that some of the sperm-chromosomes fail to develop or else stick together so that their full number is obscured. The size of the larger nuclei is, however, such that it is probable, Hinderer thinks, that they contain the triple or quadruple number of chromosomes.

These results are in accord with those of Herbst and show that after treatment with parthenogenetic agents the number of egg-chromosomes is increased. Both observers think that some of the paternal chromosomes are lost. It may appear therefore that the large nucleated plutei are false bastards in the sense that they contain only or largely maternal chromosomes. The cytological evidence fails to show explicitly that any of the paternal chromosomes are present in them in later stages although in favor of such a view it is claimed both by Herbst and by Hinderer that these large nucleated plutei show specific traits of the paternal type. From the evidence at hand, it must be assumed that whenever paternal chromosomes remain they have an influence on the pluteus.

Landauer ('22) has also found that by exposing the eggs of Spaerechinus granularis to a weak solution of ammonia in sea water (100 ccm. sea water—2 ccm. $\frac{1}{10}$n NH_3) for different intervals ($\frac{1}{4}$ to $1\frac{1}{2}$ hours), and subsequently fertilizing them with normal sperm of Strongylocentrotus (Paracentrotus) lividus, the resulting plutei are more like the maternal type than are the ordinary hybrids. Landauer has made a very thorough study of the changes in eggs subjected to this preliminary treatment with ammonia. Under its influence one, two or even three monasters appear in some of the eggs, in other eggs no changes take place in the nucleus. The appearance of monasters means that the chromosomes have divided once or twice, or three successive times before development begins. This accounts for the change in size of the resulting nuclei of the undivided eggs whose volumes are 1 : 2 : 4 : 8, according to whether no division, or one, or more have taken place. An examination of the chromosome number shows that they have divided at each monaster stage. Consequently the diploid or triploid number is present except in so far as one or more chromosomes may fail to separate. When these thicker double chromosomes are counted as two,

their number corresponds with expectation. When the sperm enters—monospermic fertilization is the rule—the sperm-nucleus unites with the egg-nucleus and the chromosomes of the combined nuclei enter together into the first polar spindle. The paternal chromosomes divide regularly at each mitosis. Only rarely are paternal chromosomes left outside the spindle.

Landauer finds in these eggs no evidence of suppression of paternal chromosomes. The nuclei of the regular hybrid plutei are somewhat larger than those of the paternal species. The nuclei of the triploid, tetraploid, hybrid plutei are larger than those of the normal hybrids in agreement with the larger number of chromosomes present.

The calcareous skeleton of the triploid and tetraploid plutei has, both as to size and to form, a remarkably strong resemblance to the skeleton of pure Sphaerechinus plutei in contrast with the normal (diploid) hybrid pluteus which on the average stands exactly midway between the parental types.

These results like others of their kind may be interpreted to mean that the ammonia solution initiates the beginning of the parthenogenetic development that involves, however, visible changes only in the nuclei. No fertilization membrane is thrown off and consequently the eggs may be entered by a sperm. Since only one sperm enters it appears that surface changes in the egg must take place so that other sperms are prevented from entering. This result may be significant if, as seems to be the case, the block to development may be removed from the nucleus without producing cortical changes. Since ammonia solution does not lead to complete parthenogenetic development, the result may also be interpreted to mean that cortical changes have been started, sufficient to remove the block inhibiting the division of the chromosomes (resolution of the egg nucleus) but without altering the surface to the extent of interfering with subsequent fertilization.

CHAPTER XXIV

MENDELIAN INHERITANCE OF EMBRYONIC AND LARVAL CHARACTERS

The modern work on heredity, based on Mendel's laws and their later expansion, has dealt almost exclusively with adult characters, yet there is a fair number of cases on record in which the characters relate to embryos, or to larval stages. These also have been found to follow Mendel's laws.

Much of the work of embryologists on hybridization had been done before Mendel's laws were recovered, and, like most of the work that preceded Mendel, had been made with species-crosses, and consequently had failed to give much insight into the laws of inheritance. Furthermore, since the observations were never carried to the second generation, practically all this work has no very fundamental importance for the problems of heredity. Even after the methods, that geneticists had found to be essential for the study of inheritance, were clearly understood, the older type of work, in which crosses were made between widely different species of animals often belonging in fact to different families, or even orders, continued to be done by embryologists. It goes without saying that little or nothing has been contributed by such a procedure to the study of heredity, partly because the problems involved are too complex, and partly because later generations were not studied, indeed seldom procurable. Nevertheless other results of importance have arisen from this embryological work, and a few that have real significance. Some of these may be briefly enumerated here, but will be dealt with more in detail in later pages. First in importance perhaps is maternal inheritance since it serves to illustrate the impress that the cytoplasm of the egg has received from the inherited chromosome complex before the elimination of half of the chromosomes. For example, reciprocal fertilization between two types—whether species differing only in one or more

Mendelian factor pairs makes little difference—has shown that, in the early stages at least, the embryo responds to the cytoplasmic influences present in the egg at the time of maturation, i.e., before the elimination of the polar bodies. At maturation the chromosomes, carrying the genes that have given the distinctive features to the egg, may have been eliminated.

The time at which the sperm begins to produce its influence on the egg has also been studied by embryologists in connection with the influences already present in the cytoplasm.

An important problem, intimately related to the foregoing ones, still remains to be solved, and a way to study it has been found by embryologists. I refer to the influence of a nucleus derived from one type on the isolated cytoplasm of another type. At the time when Boveri tried to answer this question by fertilizing a non-nucleated fragment of one species of sea-urchin by a single sperm of another species, it seemed that this was the most promising line of attack, but with the advent of Mendelian methods, the problem solved itself along other lines. The results have shown that the cytoplasm responds ultimately to the genes irrespective of the kind of egg from which the cytoplasm has come. Only minor matters remain to be cleared up, such as the time required for the genes to bring about their effects, and whether there are certain further cytoplasmic differences that characterize species (if there are any such cytoplasms) that are independent of genic influences. The more profound problem as to the way in which the genes affect the cytoplasm remains unsolved, and calls for a combined attack by both embryologists and geneticists.

Concerning the influence of unbalanced sets of chromosomes, the most recent work on genetics will have a restraining and beneficial influence on embryological opinion.

In the account that follows, the hybridizing work, that has been done from the modern genetic standpoint, is first given. While these results are not different from other accounts of Mendelian inheritance they may serve, by contrast, to make evident the difficulties when crosses are made between species.

Inheritance of Color in Silkworm Larvae

The caterpillars of the silkworm moth (Bombyx mori) have furnished the best evidence of Mendelian inheritance in larval stages. Many races of silkworms are cultivated. The cater-

pillars show racial differences, especially in their color markings, and in the color and shape of their cocoons. The heredity of more than a dozen different types of caterpillars and of several kinds of cocoons has been worked out. In addition, the color of the eggs and young embryos enclosed in the eggs have furnished important evidence of maternal inheritance. The number of broods produced each year has also been shown to be maternally inherited.

Toyama ('06) found that when a "striped" or zebra race is crossed to a common pale race, all the resulting caterpillars are striped. If these are reared and the moths inbred, there are produced three striped (1376) to one pale (417). The two races differ by one factor difference, and the results are like those found by Mendel in peas, and explicable on the assumption that in the germ-plasm of the hybrid, the element for striped (that comes from one parent) separates from the element for pale (that comes in from the other parent). Half the eggs of such a hybrid contain the striped element and half that for pale.

Similarly in the hybrid male, half the sperm carry the element for striped and half for pale. Chance meeting of any egg by any sperm will give one pure striped to two hybrid striped to one pale, i.e., 3 striped to 1 pale. Toyama also showed that when a race producing yellow cocoons is bred to a race with white cocoons, the offspring (F_1) produce yellow cocoons. If the F_1 moths are inbred, they produce three yellow cocooners to one white.

Toyama made crosses in which both larval and cocoon characters were involved. A pale race spinning yellow cocoons was bred to a striped race spinning white cocoons. The offspring were striped and produced yellow cocoons. When these were inbred they produced 9 striped, spinning yellow cocoons; to 3 striped, spinning white cocoons; to 3 pale, spinning yellow cocoons; to 1 pale, spinning white cocoons. These are the characteristic Mendelian ratios when two pairs of characters are present in a cross. If the members of each pair separate (segregate) in the hybrid, and if the separation of one pair is independent of that of the other pair, there should be produced in equal numbers four kinds of eggs and likewise the same four kinds of sperm, namely, striped yellow, striped white, pale yellow and pale white. If any one of these four kinds of eggs be

fertilized by any one of the four kinds of sperm there will be 16 possible combinations. If one remembers that striped dominates pale (when both are present) and yellow dominates white, these 16 combinations fall into four classes in the ratio of 9 : 3^a : 3^b : 1 as shown in the square below, where the dominant characters are italicized.

Eggs / Sperm	Striped Yellow	Striped White	Pale Yellow	Pale White
Striped yellow	*Striped yellow* *Striped yellow* (9)	*Striped* white *Striped yellow* (9)	Pale *yellow* *Striped yellow* (9)	Pale white *Striped yellow* (9)
Striped white	*Striped yellow* *Striped* white (9)	*Striped* white *Striped* white (3^a)	Pale *yellow* *Striped* white (9)	Pale white *Striped* white (3^a)
Pale yellow	*Striped yellow* Pale *yellow* (9)	*Striped* white Pale *yellow* (9)	Pale *yellow* Pale *yellow* (3^b)	Pale white Pale *yellow* (3^b)
Pale white	*Striped yellow* Pale white (9)	*Striped* white Pale white (3^a)	Pale *yellow* Pale white (3^b)	Pale white Pale white (1)

Later ('12) Toyama discovered that there is a race that is recessive for white cocoon color and another race that is dominant for white cocoon color. If the recessive is crossed to a race with yellow cocoons the offspring produce yellow cocooners. If these F_1's are inbred they give 3 yellow cocoons to 1 white. On the other hand if a dominant race is bred to a yellow-cocoon race the offspring produce white cocoons. If these F_1's are inbred the expectation is 3 white cocooners to 1 yellow.

Caterpillars that spin yellow cocoons have yellow colored blood; those that spin white cocoons have colorless blood. The color of the blood can be seen through the skin, particularly on the inside of the abdominal legs. The caterpillars in the last cases could be separated according to blood color as well as by the cocoon color. The outcome is the same.

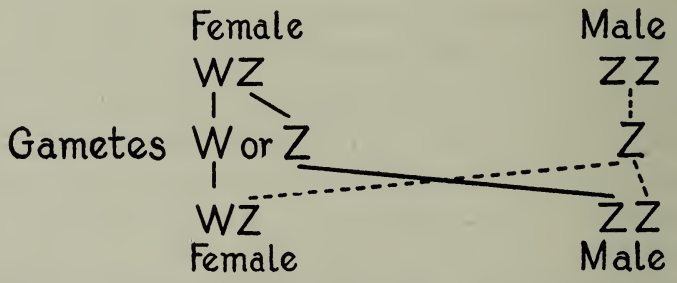
In addition to the kinds of caterpillars of the silkworm moth described above, there are several other characteristic types whose inheritance has been studied by Tanaka ('13, '14, '16). The factors of four of these (S, Z, M, N) were found to be dominant to four other types taken as allelomorphs (s, z, m, n), the latter when present alone producing a "plain coat." Later, Tanaka found that three of these (S, N, M) represent multiple allelomorphs; that is, each is a modification of the same factor, hence, only two of them can occur in the same animal at the same time (thus SN or SM or NM). Another factor Q (quail) is also said to be an allelomorph of S and M, hence of N also. The coloring of the larva homozygous (pure) for two of these factors (SS or QQ) is somewhat different from that of the combination of two of them (SQ for example). Tanaka also made the interesting discovery that the factor for yellow cocoon color (Y) is linked with each of these four allelomorphs. This means that when from one parent one of the factors (S, M or Q) enters a cross combined with yellow cocoon, and from the other parent there enters another one of the caterpillar colors combined with white cocoon (two pairs of factors) there is not found in the second generation a 9 : 3 : 3 : 1 ratio, but a modification of the ratio due to the combination that went in together (SY on one side and My on the other) tending to remain together in the second generation, producing there a higher percentage of each combination that went in than expected on free assortment of the two pairs (S and M and Y and y). This phenomenon, known as linkage, is also often met with in crosses when adult characters are studied. It finds a rational explanation in the view that linked characters are carried in the same chromosome. Thus, the gene (S) for striped (not Toyama's "striped") and yellow cocoon (Y) character are carried in one chromosome and the character moricaud color (M) and white (y) in the corresponding chromosome of the other parent. These two chromosomes meet in the hybrid and separate when the germ-cells of the hybrid mature, giving two kinds of eggs (SY and My) and two kinds of sperm. If nothing further than this happened in the hybrid, then chance union of any egg with any sperm would give only the following combinations, 1 SYSY (striped yellow) : 2 SYMy (striped yellow) : 1 MyMy (moricaud white). The combinations that went in together would come out together, or, in other words, there would

appear in F_2 only the two grandparental types in the ratio of 3:1. But the situation is not quite so simple as this, because recombinations of the characters that went in also appeared in the second generation, although, as stated above, not in the numbers expected (9:3:3:1) on free assortment of the two pairs. The explanation of this situation is also clear to-day; for, it has been shown that even when hereditary factors enter the cross in the same chromosome there may be an exchange of factors in the hybrid between this chromosome and its mate. This is the familiar phenomenon of crossing over. Since the interchange is not as free as when the genes in question lie in different pairs of chromosomes, the numerical results are correspondingly altered. Tanaka has shown in the female silkworm that no crossing over takes place (complete linkage) while, in the male, crossing over does occur to some extent (partial linkage).

The inheritance of a few characters in other species of moths has also been studied. Goldschmidt ('21) records that the black type of caterpillar of the nun (Lymantria monacha) is dominant over the light type; the two characters behave as a single pair of Mendelian units. In an earlier account ('17) of crosses between different races of the gypsy moth (Lymantria dispar), a more complex situation is described by Goldschmidt. The different races of the moth, spread over Europe and Asia, have different races whose caterpillars show constant differences of pigmentation. The F_1 offspring are "about intermediate," the F_2 generation breaks up roughly into 3 light (medium) to 1 dark, if young stages of the caterpillars are alone considered. This was interpreted to mean that the degree of coloring in the different races is due to a series of multiple allelomorphic factors with different powers to produce pigment. As yet no sufficient data have been given to establish such a view. Goldschmidt concluded further that the factors in question are only different quantitative amounts of the same factor. Speculating further, along these lines, Goldschmidt assumed that by selection of the fluctuations of the factors (genes) in a plus or a minus direction a new mean could be established. The evidence that he appealed to was quite insufficient to establish such a conclusion which has been shown not to be true in other cases where a more critical test has been applied. In addition to the difference between young larvae Goldschmidt also found that characteristic changes

in the pigmentation of the caterpillars of different races take place as they pass from molt to molt. Light caterpillars remain light through the entire larval life in some races. In other races the caterpillars may become darker in some cases than in others. Medium light caterpillars of differing degrees may also change to dark. These differences were also ascribed to differences in "quantity" of the allelomorphic genes.[1]

In the adults of several animals (man, fish, flies and moths) there is a special type of Mendelian heredity in which the character in successive generations follows the known distribution of those chromosomes connected with sex determination. There is also a case of this kind in the caterpillar of the silkworm moth (Bombyx mori) where Tanaka ('22) found that one of the several types of translucent worms behaves in inheritance as though the character were carried by one of the Z-chromosomes. In this moth, as in others of this order, the female is supposedly heterozygous for the Z-chromosome (WZ) and the male homozygous (ZZ). Thus:

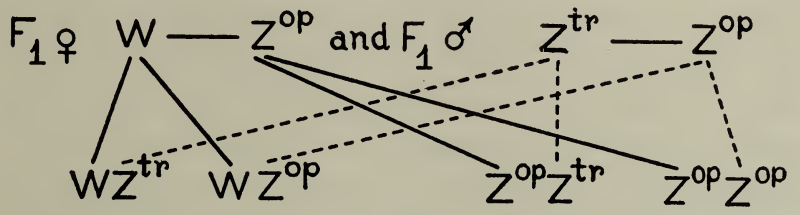


The translucent character is recessive to the normal or opaque skin—the difference depending on the presence of white granules in the more opaque skin.

If a female moth of a race with translucent larvae (WZ^{tr}) is mated to a male of a race with opaque larvae (Z^{op} Z^{op}), the daughter caterpillars (WZ^{op}) are opaque like the father, because they get their single Z^{op} from him; the sons (Z^{tr} Z^{op}) also are

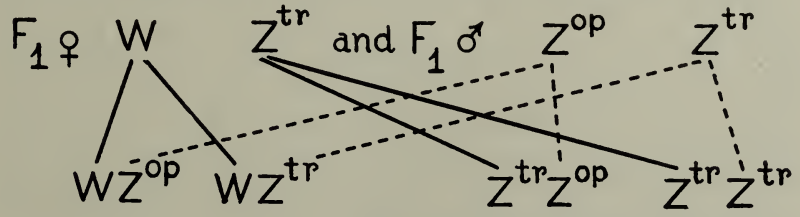
[1] In some wild races of the gypsy moth (Lymantria dispar) the caterpillars have a white streak along the dorsal midline; in other races the caterpillars have a broad black band along the back. When moths of these races are crossed the first generation caterpillars are black. When the F_1 moths are bred, the F_2 caterpillars are black or striped in the ratio of three black to one striped. This result was obtained by Klatt ('19) and confirmed by Baltzer ('20).

opaque, because the opaque character (Z^{op}) dominates the translucent character (Z^{tr}). If the moths from these F_1 caterpillars are inbred, half the F_2 daughter caterpillars are translucent, half are opaque and all the F_2 sons are opaque. Thus:



The ratio is 1:1:2. It is apparent that the translucent character of the grandmother's race is transmitted to half the granddaughters and to none of the grandsons, although half of the grandsons carry one factor for translucent. This redistribution of the character conforms to expectation if the pair of genes involved is borne by the Z-chromosomes of the two races.

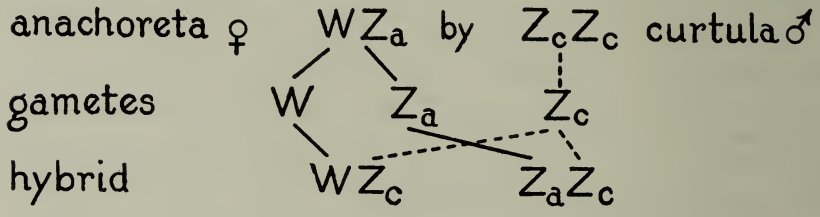
The converse experiment is equally instructive. If a female moth of a race with opaque larvae (WZ^{op}) is mated to a male of a race with translucent larvae (Z^{tr} Z^{tr}) the daughter caterpillars (WZ^{tr}) are translucent like their father, because they get their single Z^{tr} from him, and the sons (Z^{op} Z^{tr}) are opaque because of the dominance of the opaque character. If the moths from these F_1 caterpillars are now inbred half the daughter caterpillars are opaque, half are translucent, and half the male caterpillars are opaque, half are translucent. Thus:



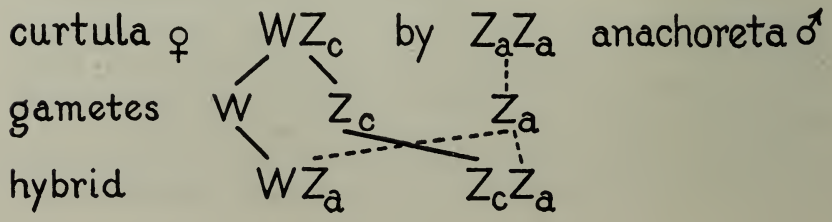
The ratio is 1:1:1:1. Here the translucent character of the grandfather is transmitted to half of the granddaughters and to half of the grandsons. This redistribution of the character conforms again to expectation based on the behavior of the chromosomes.

Federley ('11) has described a case of sex-linked inheritance in a species-cross between the moths Pygaera anachoreta and

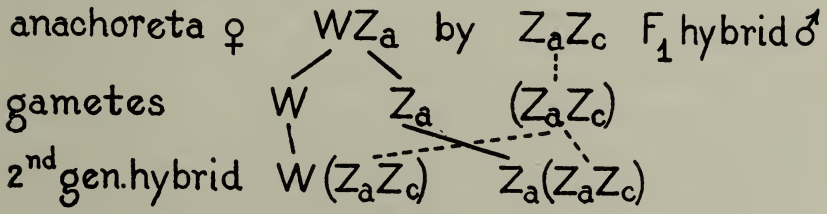
P. curtula. The male and female caterpillars are alike in each species, but the caterpillars of one species are different from those of the other. When P. anachoreta is the mother and P. curtula the father, the male and the female hybrid caterpillars, after the first molt, are markedly different. The difference involves the form, color and marking of the two kinds of individuals. The male hybrid caterpillars are closely similar to the caterpillars of the maternal race (anachoreta), while the hybrid female caterpillars resemble those of the paternal race (curtula). The explanation of this result is apparent if the character differences are due to genes carried in the Z-chromosomes; for the daughter gets her single Z-chromosome from her father, which she resembles, while the son gets one from each parent. If now the maternal Za (anachoreta) carries a gene (or genes) dominant to the gene (or genes) in the Zc of the father (curtula), the son will resemble the mother because *ex hypothesi* she carries the dominant gene or genes.



The reciprocal cross bears out this interpretation. Thus, if P. anachoreta is the father and P. curtula the mother, all the offspring are alike. In this case the daughter again gets her single gene Za (dominant) from her father, while the son also gets this dominant gene (Za) from his father, but also the recessive gene (Zc) from his mother. Here, then, the son and daughter are both alike because the daughter carries only the dominant chromosome and the son carries both—the dominant one determining his character. Thus:



The analysis is further borne out by back-crossing the hybrid male to one or the other parental races. (It was not possible to make an F_1 cross, owing to the sterility of the female hybrid.) Federley succeeded in making such crosses. The results can not be explained by the ordinary extension of the formula, because, as Federley showed, the usual reduction of the chromosomes at the maturation division does not occur in the male hybrid. On the contrary, each spermatozoön carries the diploid number —one set of maternal and one set of paternal. When these sperm fertilize the eggs of a female of either original stock (curtula or anachoreta) the resulting offspring are triploid. Nevertheless, the relation of dominance and recessiveness assumed for the first generation crosses will explain the observed results, provided two doses of the recessive gene (two chromosomes carrying these genes) do not affect the dominance of the other genes (the other chromosome carrying these genes). Thus:



The caterpillars of some moths have two (or more) color forms in the later stages. Weismann ('76) had speculated as to the interpretation of these types. Federley ('16) reared green and dark caterpillars, and mated the moths that came from them. Green female to green male, green female to dark male, dark female to green male and dark female to dark male. All the offspring were dark caterpillars. The results show that the difference is not genetic but environmental. The external factor that causes the change was not discovered. On the other hand Gerould ('21) has recently found a new larval color type of the common clover butterfly (Colias philodice) that behaves in its inheritance as a Mendelian recessive. This blue-green caterpillar appeared as a mutant of the normal yellow-green type. As in several other mutant characters in silkworm moths this one also is connected with an alteration in the color of the blood. In the normal caterpillar of this species, the color of the blood makes the caterpillar yellow-green. Correspondingly, the blood

of the mutant is blue-green; it shines through the skin after the first molt. Poulton pointed out ('85, '93) that in plant-eating caterpillars one green pigment in the blood is derived, with only a little change, from the chlorophyll of the food plant. It is the way then in which this change is affected by the genetic make-up of the mutant caterpillar that makes the color of the blood different from that of the normal caterpillar.[2]

In the group of moths and butterflies the sex chromosomes are represented by the formula WZ ♀, ZZ ♂. In other groups of insects another formulation holds, namely, XX ♀ and XY ♂. Here sex-linked inheritance is the same in principle as in moths, if the X's carry the sex factors. There are many adult characters of insects that show this form of sex-linked inheritance, and there is one case at least of a larval character that is inherited in the same way. In the vinegar fly there is a race which carries in one of its X-chromosomes a Mendelian factor that produces a tumor in the larva, and any larva that carries this factor alone dies. The inheritance of the tumor may be illustrated by an example. Half of the stock females carry one lethal factor in one of the X-chromosomes, the other chromosome carries the normal partner (allelomorph) of this factor, and since the normal factor is dominant the female does not perish in the larval stage. She produces two kinds of eggs after the extrusion of one or the other X in the polar bodies. One X carries the lethal factor for the tumor, the other X its normal partner. If the eggs of such a female are fertilized by the sperm of a normal male—half of whose sperm are X-bearing, and half are Y-bearing—four possible kinds of embryos are expected. If the lethal-bearing egg is fertilized by the X-sperm, a daughter like the mother, is produced; if the egg bearing the normal X is fertilized by an X-sperm, a normal daughter is produced. The former is like the mother and transmits to half of her offspring the lethal factor, the other daughter is entirely normal and never transmits the gene. If the lethal-bearing egg is fertilized by the Y-sperm, the sons, so produced, die because each contains

[2] Hein ('24) has recently found three types of larvae of the meal worm (Tenebrio molitor) whose heredity shows that they are represented by three allelomorphic genes. Tower ('10) has shown that two larval types of the beetle, Leptinotarsa signaticollis, are represented in the germ-material by a pair of Mendelian genes.

only the maternal X with its fatal contribution. If the other kind of egg (bearing the normal X) is fertilized by a Y-sperm, a normal son is produced that does not transmit the gene to any of its descendents. The sex ratio has been changed, owing to the death of half the sons. The result is two daughters to one son.

In the sex formulae that have been here used, the WZ-ZZ and the XX-XY types, the W-chromosomes in the former and the Y-chromosomes in the latter have been ignored because experience has shown that they carry almost no genes that affect the Mendelian results.[3] It is not to be inferred that even in these types no factors are carried by the W- or Y-chromosome. It has, in fact, recently been shown that the heredity of certain adult characters can only be explained on the view that such factors are carried by the Y-chromosome, and certain results of Goldschmidt on the gypsy moth have also been accounted for by him on the assumption that the W-chromosome carries certain Mendelian factors.

Thus it has been shown by Schmidt ('20), and confirmed by Winge ('22, '23) that in the fish Lebistes a character peculiar to the male is carried by the Y-chromosome, or at least the inheritance of this character is explained if its distribution is the same as that of the Y-chromosome. Since the Y-chromosome never gets into the female line it follows that the gene is never carried by the female and is transmitted only from father to son. It differs from the other type of sex-linked inheritance in this important respect, since in the XX-XY type the X-chromosome is shuffled back and forth between the sexes, as is the Z in the WZ-ZZ type.

Aida ('21) has also found in another fish Amplocheilus that certain characters are carried by the Y-chromosome and both Winge and Aida show that crossing-over may take place between the Y- and X-chromosome. It would seem to follow if the sex mechanism depends on a constant relation between the X, or the Y and the other chromosomes, that crossing over between X and Y would soon make them alike and destroy their relation in

[3] In Drosophila melanogaster a male lacking the Y-chromosome is sterile. Otherwise he shows the normal characters of his sex. There has also been found one character in the Y-chromosome that is the normal allelomorph of a mutant factor in the X-chromosome. (Stern '26.)

the sex-scheme. This would be true only if more than one sex factor exists in the sex chromosomes; for if only one gene for sex is present it might be shuffled back and forth indifferently without affecting the sex ratio. Then one of the two sex chromosomes which contained the Y-gene would, by definition, become Y. It can only be surmised, in case there is more than one sex factor in the sex chromosomes, that crossing over would occur only in that part of the X that does not contain the sex factors. The failure to cross over might possibly be due to difference in length of the X and Y, or to some other relation interfering with crossing over in one end or in some part. This suggestion may not seem so fanciful if it is recalled that in Ascaris it has been found that the X-chromosomes are attached to another pair of chromosomes, that would correspond, therefore, to the supposititious X and Y of the fish. More recent genetic work (Gordon '26), on another fish (Platypoecilus maculatus) of the same family has furnished evidence that in this case the female is the heterogametic sex.

Inheritance of Color in the Chick

The inheritance of the color of the down of newly hatched chicks presents some unique problems. The color of the down may be and generally is quite different from that of the adult fowl, yet a certain down color is associated with definite adult colors. In some races of poultry the color of the down is uniform or nearly so, while in other races there is a characteristic down-pattern that bears no obvious relation to the pattern of the adult bird. The inheritance of these characters has not been fully worked out, but enough has already been done by Bateson ('02), Bateson and Punnett ('06), Goodale ('09), and Punnett ('23) to show that the inheritance is Mendelian. A few typical cases may be given.

The down of white-breeds, whether the white is the dominant or the recessive, is yellowish. If a dominant white-breed is crossed to a colored-breed the down of the F_1 chicks is yellowish, although it may be slightly ticked, i.e., it may show a few, colored down-feathers. If a recessive white is crossed to a colored race the chicks have colored down. The actual color may depend on the color factors carried by the recessive white if these factors are dominant to those of the P_1 colored-breed.

Buff races have buff chicks; black races have black chicks;

blue adults come from blue chicks. Chicks of the Brown Leghorn race and of Game Bantams, both of which races approximate to the wild-type, Gallus bankiva, are brown-striped, i.e., they have a brown pattern on a buff background. Barred birds, such as Plymouth Rocks, have black chicks less intense black than those of black breeds, and they have also a yellowish-gray patch on the back of the head. Other "barred" races, such as the Gold Pencilled Hamburgs, have striped chicks which are less conspicuously striped, however, than those of Brown Leghorn chicks. The striped chicks of another "barred" race, the Campines, have very wide stripes, etc.

The inheritance of these characters of the chick follows closely the kind of inheritance shown by the adult bird of the races to which they belong, and, for the most part at least, may be supposed to be due to the effects of the same color factors acting on younger stages in which their effect may be outwardly quite different from the effects of the same factors on the adult birds. However, the work has not progressed sufficiently as yet to exclude the possibility that there may also be specific factors that affect primarily the color of the chick and to a less extent that of the adult.

When certain races are crossed, more particularly where one race is silver and the other gold, the inheritance is sex-linked and follows the same rule as that for sex-linked characters of moths. The female has one Z-chromosome and the male two Z's which carry these contrasted characters. The chicks show the same kind of inheritance as the adults, and since the difference is apparent at hatching it enables the breeder to pick out at once the F_1 males from the females if a suitable cross has been made. Thus, according to Punnett, when a Light Sussex hen is bred to a Brown Leghorn cock the silver male chicks are markedly different from the gold female chicks.

CHAPTER XXV

THE DEVELOPMENT OF SPECIES HYBRIDS

The term cross-fertilization has several meanings. As used by botanists for hermaphroditic types, it applies to those cases where the pollen of one plant fertilizes the ovule of another plant of the same species. The term has often been applied, especially by zoologists, to crosses between two varieties, or even between two species; but both botanist and zoologist generally use the term hybridization when two species are crossed. Even in Mendelian heredity the offspring of two individuals, differing in one or more characters, are frequently called hybrids.

It is sometimes stated that "species" do not hybridize, or, if they do, that the offspring are sterile; but there are many cases in which offspring are produced, both in animals and plants, where there can be little question that the two individuals involved would be ranked as distinct species. For example, the horse produces with the ass the sterile mule, and the cow and buffalo produce the cattalo, which is partly fertile.

The reverse criterion is more valuable; for, groups that do not cross may be held to be distinct species on account of this test, but it is generally conceded to-day that definitions as to what constitutes a species are largely arbitrary, and change from group to group. It is clear, that there is only a very loose relation between the occurrence of cross-fertilization and species differences.

The sterility of the hybrid, when such is produced, has been said to be a more severe test as to whether two groups deserve the title of species, but here also the distinction often breaks down. To-day little weight is attached to these attempts to find an absolute definition as to what constitutes a species, for we have come to realize that the grouping of individuals into species is little more than a convenient method of arranging them for purposes of classification.

Most of our information concerning the early stages in the development of animal hybrids is confined to amphibians, sea-urchins and Teleostean fishes.

Anuran Hybrids

The first attempt to cross different species of frogs was made by Rusconi (1840). He tried to fertilize the eggs of the green frog with the sperm of a toad. The eggs cleaved, but went no further. De l'Isle (1873) made many attempts to cross different species without obtaining even cleavage stages, except in the case of Bufo vulgaris and Bufo calamita, where the eggs developed into tadpoles, which died without becoming frogs. Lataste (1878) failed in an attempt to hybridize different species of urodeles, but successfully crossed the two toads Pelobates fuscus (female) and P. cultripes (male). The embryos that developed were abnormal.

Many attempts to cross anurans were made by Pflüger (1882). Eggs of Bufo cinereus were inseminated by sperm of Rana fusca. Segmentation took place, but the eggs died in the blastula stage. Reciprocally the result was less successful. Eggs of Rana esculenta segmented quite regularly when fertilized by the sperm of R. fusca, but came to a standstill in the later cleavage stages. Reciprocally no results were obtained. Born ('83) made some of these same crosses with similar results; also crosses between other species of frogs, some of which gave segmentation stages. Hybrid tadpoles were obtained in only two cases. Born found that cleavage took place much oftener when concentrated sperm was used. It is probable, from results obtained later by others, that the cleavage that he observed was due to polyspermy. Pflüger drew the conclusion from his crosses that spermatozoa with pointed heads, or with smaller heads, more frequently enter the eggs of other species than do sperm with larger and blunter heads. This difference might be supposed to be connected with the ability to pass through the jelly around the eggs, rather than with a difference in power to unite with the egg. No direct observations have been made to settle this point.

Chromosomes of Hybrid Amphibia

Gunther Hertwig made the discovery ('13, '18), that when the eggs of the frog Rana esculenta are fertilized by the sperm of the toad, Bufo viridis, the tadpole that develops is like that of Rana esculenta (false bastard, or hybrid). Hertwig gives the following explanation of this result. The sperm of the toad, entering the egg, starts the development, but takes no further part in it. The haploid nucleus of the frog's egg is activated, and by division gives rise to all the nuclei of the embryo, hence the resemblance of the tadpole to that of the maternal species. The details of these experiments are as follows:

Hertwig observed that after insemination with the toad's sperm, only 10 to 25 per cent (A), of the esculenta eggs segmented at the same time as did the normally fertilized (control) eggs of the frog. Most of the cross-fertilized eggs (B), that had not segmented at that time, began to divide when the normal control eggs segmented into four cells. They divided into two cells, but more or less irregularly. Some eggs of the cross-fertilized set (C), still remained undivided, and only later after an hour or two cleaved, but cleaved for the most part quite irregularly. All eggs that divide irregularly die before they reach the gastrula stage, while those that divide into two equal, or nearly equal cells, may develop into tadpoles.

The eggs (A) that cleaved into two at the normal time, gastrulated in the regular way, and gave rise to typical dwarf tadpoles with swollen bodies due to accumulation of water in the body cavity. They remained alive from two to three weeks. These had haploid nuclei.

Those eggs of the other set (B) that cleaved into two also gave rise to tadpoles nearly all of which showed white necrotic spots on the surface. Except in cases where these necrotic areas led to their death, the tadpoles developed much better than those of the former set. They did not swell up with water, and could be kept alive almost to the time of metamorphosis. They were exactly like the normal, control tadpoles of the maternal species. They had the same size, and showed all the characters of Rana esculenta.

The differences in the development of these two sets of tadpoles (A and B) were found by Hertwig to be correlated with a

difference in the number of their chromosomes. The former (A) have the half-number of chromosomes, the latter the full number. The set (A), that divided at the same time as the control, did so with the half-number of chromosomes present in the egg-nucleus, while the other set (B), that did not divide until the normal control divided into four, must have undergone one mitotic division without an accompanying cytoplasmic division. At the next division period of these eggs, the whole number of chromosomes would then be present. This explanation of the doubling, while not resting on actual observation of the stages in question, would in itself be probable from what is known in other eggs under somewhat different conditions. Hertwig, however, has further demonstrated that the cells of the body of the second set of tadpoles (B), contain the double number of chromosomes; and the nuclei are the same in size as those in the normal (diploid) embryos. It is interesting to note that, despite the early necrosis, these tadpoles (B) with the full number of chromosomes develop better and go farther than do the tadpoles with the half-number of chromosomes. Moreover, the former with the whole set are full-sized tadpoles; the latter with the half-set are dwarfs.

A remarkable case of a tetraploid individual was met with. A female (Rana esculenta) had been inseminated with the sperm of Bufo viridis. Out of two hundred eggs that had been inseminated, 52 were fertilized, as shown by their rotation within the jelly. Of these, 31 eggs (60 per cent) cleaved (A^1) into two equal blastomeres at the first division period of the normal control. Of the remaining eggs (B^1) a very few divided irregularly when the control was in four cells, but none regularly. These died soon afterwards.

The former set of eggs (A^1) gastrulated normally, and developed into tadpoles. Fifteen of these, that were alive after eleven days, were not like normal tadpoles; they were weak and pathological. Later all were preserved for histological study. The somewhat exceptional behavior of these eggs (time of cleavage, character of larvae, etc.) suggested that the female must have been different in some way from ordinary females. If she were tetraploid her eggs would be diploid after extrusion of the polar bodies. Such a diploid egg, started to develop by the sperm of the toad, would contain diploid nuclei. If this is the correct interpretation, then, any eggs of this female that had

been fertilized by sperm of the same species should be triploid. There were, in fact, some embryos of this kind, and chromosome counts verified their triploid character.

The diploid number of chromosomes for Rana esculenta was found by Gunther Hertwig to be 24. Rana fusca also has 24 chromosomes according to several observers. The number present in the tissues of the tadpoles from eggs of the exceptional female (R. esculenta), whose eggs had been fertilized by sperm of Rana esculenta, was found to be 36, which is the triploid number.

Hertwig made measurements of the nuclei in cells of older tadpoles of the different kinds obtained in his crosses. A comparison of their volumes substantiated the conclusion already given, provided the size of the nucleus is assumed to be in proportion to the number of chromosomes it contains.

Echinoderm Hybrids

A large number of hybridizing experiments have been made with echinoderms, especially between species of sea-urchins. Since most of the species can be crossed, and since the characters of the larvae (plutei) of different species are quite marked, and appear very early, the echinoderms furnish excellent material for the embryological study of hybridization, at least for the larval stages.

The earliest experiments in crossing species of sea-urchins were those of Marion (1873), who obtained plutei from Strongylocentrotus lividus and Sphaerechinus granularis. Another French zoologist, Koehler ('83) crossed five species including spatangoids. He found that while some of the combinations failed to develop at all, others reached the blastula stage, and still others went as far as the pluteus stage. Stassano ('83) crossed four species of echinoderms. O. and R. Hertwig ('87) made numerous crosses, but did not study in detail the character of the hybrids. They found that the crosses were more easily made when the eggs had stood some hours in sea water than when the eggs were new, an observation later confirmed by Tennent.

A list of the hybrid combinations between different echinoderms, sea-urchins, star fish, brittle stars, and crinoids is given in the following table, (Table XXVII) taken from one of Tennent's papers ('11).

TABLE XXVII

Successful Echinoderm Cross-Fertilizations

Arbacia punctulata ♀
× Mellita pentapora ♂ (Tennent).
× Moira atropos ♂ (Tennent).

Arbacia pustulosa ♀
× Dorocidaris ♂ (Vernon).
× Echinocardium ♂ (Stassano).
× Echinus ♂ (Driesch, Stassano, Vernon).
× Sphaerechinus ♂ (Driesch, Hertwig, Stassano).
× Strongylocentrotus ♂ (Driesch, Hertwig, Vernon).

Asteracanthion berylinus ♀
× Asteracanthion pallidus ♂ (Agassiz).

Asterias forbesii ♀
× Arbacia punctulata ♂ (Morgan)

Dorocidaris ♀
× Strongylocentrotus ♂ (Vernon).

Echinocardium cordatum ♀
× Arbacia pustulosa ♂ (Stassano, Vernon).
× Echinus ♂ (Vernon).
× Sphaerechinus ♂ (Stassano, Vernon).
× Strongylocentrotus ♂ (Vernon).

Echinocardium mediterraneum ♀
× Echinus ♂ (Vernon).
× Sphaerechinus ♂ (Vernon).
× Strongylocentrotus ♂ (Vernon).

Echinus acutus ♀
× Arbacia ♂ (Vernon).
× Sphaerechinus ♂ (Vernon).

Echinus microtuberculatus ♀
× Arbacia ♂ (Driesch, Vernon).
× Echinocardium ♂ (Stassano, Vernon).
× Echinus acutus ♂ (Vernon).
× Strongylocentrotus ♂ (Driesch, Hertwig, Vernon).
× Sphaerechinus ♂ (Driesch, Morgan, Vernon).

Hipponoë ♀ (=Tripneustes).
× Cidaris ♂ (Tennent).
× Ophiocoma ♂ (Tennent).
× Pentaceros ♂ (Tennent).
× Toxopneustes ♂ (Tennent).

Mellita ♀
× Moira ♂ (Tennent).

Moira ♀
× Arbacia ♂ (Tennent).
× Mellita ♂ (Tennent).
× Toxopneustes ♂ (Tennent).

Psammechinus miliaris ♀
× Asterias rubens ♂ (Giard).

Psammechinus (pulchellus) ♀
× Spatangus ♂ (Köhler).
× Sphaerechinus ♂ (Köhler).
× Strongylocentrotus ♂ (Köhler).

Spatangus ♀
× Strongylocentrotus ♂ (Köhler).
× Psammechinus ♂ (Köhler).

Sphaerechnius ♀
× Echinus ♂ (Driesch, Hertwig, Morgan, Vernon).
× Psammechinus ♂ (Stassano).
× Strongylocentrotus ♂ (Driesch, Hertwig, Marion, Morgan, Steinbrück, Vernon).
× Antedon ♂ (Godlewski).

Strongylocentrotus lividus ♀
× Arbacia ♂ (Driesch, Vernon).
× Dorocidaris ♂ (Köhler, Vernon).
× Echinus ♂ (Driesch, Vernon).
× Psammechinus ♂ (Köhler).
× Spatangus ♂ (Köhler).
× Sphaerechinus ♂ (Hertwig, Köhler, Morgan, Vernon).

Strongylocentrotus purpuratus ♀
× Asterias capitata ♂ (Loeb).
× Asterias ochracea ♂ (Hagedoorn, Loeb).
× Asterina ♂ (Loeb).
× Chlorostoma ♂ (Loeb).
× Mytilus ♂ (Kupelwieser).
× Pycnopodia ♂ (Loeb).

Toxopneustes ♀
× Echinaster ♂ (Tennent).
× Hipponoë ♂ (Tennent).
× Holothuria floridana ♂ (Tennent).
× Mellita ♂ (Tennent).
× Moira ♂ (Tennent).

The list shows how extensively crossing has been carried out in this group. A few examples of some of the crosses will serve to illustrate the kinds of results that have been obtained.

The pluteus stage of sea-urchins appears one or two days after fertilization, and persists for some time before the very radical changes set in that transform the pluteus into the young sea-urchin. Under artificial conditions, development slows down when the pluteus stage is reached, and the plutei die in from ten to fourteen days, unless artificial feeding is resorted to.

The characters of hybrid embryos or larvae are said to be patroclinous or matroclinous according to whether they are more like those of the race or species to which the father or the mother belongs. The terms are purely descriptive. The resemblance may be due in some cases to Mendelian dominance in one or more characters; in other cases to cytoplasmic influence; and in still other cases to the presence of a haploid number of chromosomes derived from the father. Triploids may give matroclinous types of plutei. When one desires to state that certain characters of the young embryo are like those of the type from which the mother came, because the cytoplasm of the egg has a preponderating influence on the early stages of development, the term cytoplasmic influence is more discriminating.

One of the crosses that has most often been successfully made is that between two Mediterranean species of sea-urchins, Echinus microtuberculatus and Sphaerechinus granularis.

A typical pluteus of Echinus microtuberculatus is shown in Fig. 101*a*, *d*. It has a pyramidal form, with the four arms at the corners, each with an axial calcareous rod or spicule (skeleton) that extends upward from the base of the pyramid. The large mouth opens near the base of the antero-lateral arms. The digestive tract is a relatively large tube, partially subdivided by two constrictions into esophagus, stomach, and intestine. The anus opens on the ventral surface near the middle of the body, somewhat nearer the basal end. The skeleton has the following parts; a branch extends through the entire length of the antero-lateral arm, joining at its base the branch from the post-oral arm. At the point of junction on each side a long apical branch extends the entire length of the "body," ending in an enlargement at the "apex," where it joins the apical end of the other (right or left) branch. In addition, there is a short branch arising near

the union of the antero-lateral and post-oral branches, that extends across the middle of the body on its dorsal surface. These two "middle branches" may slightly overlap each other. The ciliated paths are confined to the oral aspect of the ectoderm, and extend out on to the four arms.

The pluteus of Sphaerechinus (Fig. 101*c*, *f*) has a more rounded form. Only two of its arms, the post-oral ones, are long; the antero-lateral ones scarcely project beyond the broad

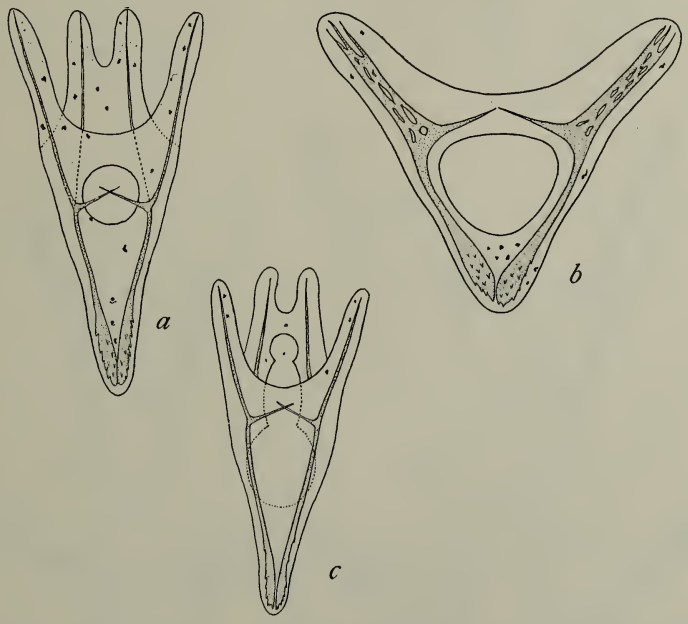


Fig. 254.—*a*, pluteus of Strongylocentrotus lividus; *b*, pluteus of Arbacia pustulosa; *c*, hybrid between Strongylocentrotus ♀ and Arbacia ♂. (After Fischel.)

oral lip. The skeleton in each anal arm is made up of three longitudinal rods connected by numerous cross-branches, producing a ladder-like structure. The skeleton in the antero-lateral arms is relatively short and is a single rod. The apical branch runs as a single rod on each side that expands and branches somewhat at the apex. There is another pair of rods in the body that lie on the anterior side, and extend backward from the antero-lateral arms. These two rods join at the blunt apex, and unite in a ring with the other two posterior rods of the body,

as shown in the figures. Short oral branches extend in the middle region across the posterior side.

The hybrid pluteus is shown in Fig. 101*b*, *e*. It is not so long as that of Echinus, or as rounded as that of Sphaerechinus. It may, with reservations, be said, therefore, to be intermediate. The arms are not as long as those of Echinus, noticeably the antero-lateral arms. The skeleton shows much variability. In the type represented in the figures it is not exactly like that of the pluteus of either parent form. On the whole, it is simpler, and more like that of Echinus, but there are two rods in the post-oral arms, and at the apex there is more branching than

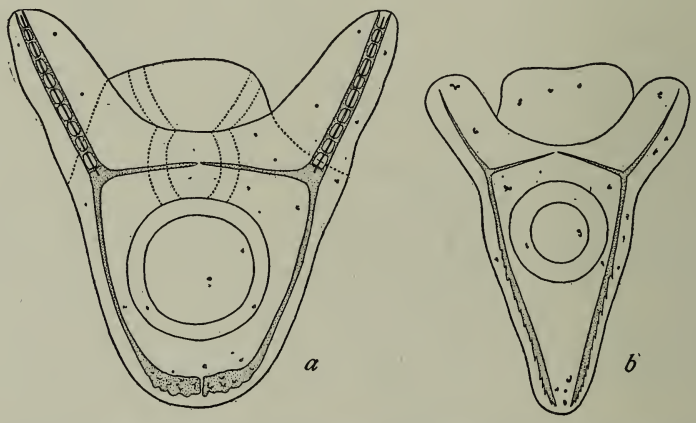


Fig. 255.—*a*, pluteus of Echinus brevispinosus; *b*, hybrid between Strongylocentrotus ♀ and Echinus ♂. (After Fischel.)

in Echinus. It is not accurate, therefore, to describe the hybrid skeleton as intermediate, for it would be difficult to determine what a true intermediate type would be, but it may, at least, be said that the hybrid shows some of the characteristics of the pluteus of each parent.

Fischel ('06) has described and figured hybrid plutei between two other Mediterranean sea-urchins, Strongylocentrotus lividus and Arbacia pustulosa. The hybrid resembles more the maternal type, with only traces of paternal influence (Fig. 254*a*, *b*, *c*).

In other crosses made by Fischel where the parental types are not so different, the hybrid larva bears resemblances to both paternal types, and it is impossible to tell whether the influence

of the egg or that of the sperm predominates. In only one of these crosses is the larva more patroclinous, namely Echinus brevispinosus eggs by Strongylocentrotus sperm, and in this case only in the young triangular stage, beyond which the larva does

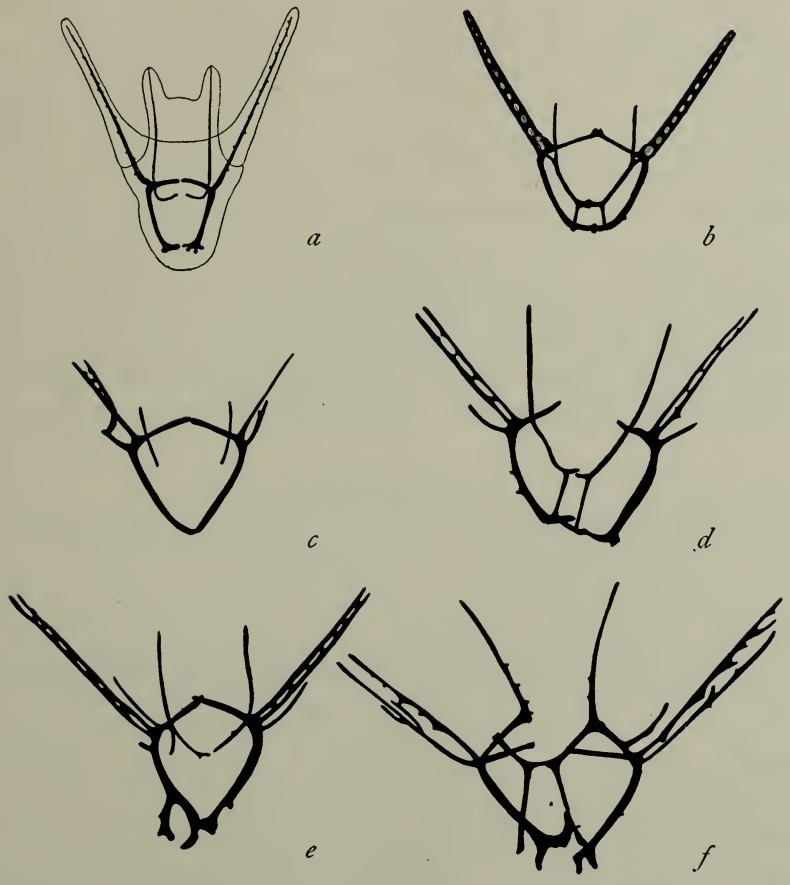


Fig. 256.—*a*, pluteus of Toxopneustes (Lytechinus) variegatus; *b*, skeleton of pluteus of Hipponoë esculenta; *c–d*, skeleton of pluteus of Hipponoë ♀ by Toxopneustes ♂ reared in alkaline sea water; *e, f*, skeleton of pluteus of Toxopneustes ♀ by Hipponoë ♂ reared in alkaline sea water. (After Tennent.)

not develop. The reciprocal cross (Strongylocentrotus eggs by Echinus brevispinosus sperm), is nearly matroclinous (Fig. 255*b*). MacBride ('11, '12) fertilized the eggs of a spatangoid, Echinocardium cordatum with the sperm of Echinus esculentus. He

found that the apical spike was absent in the hybrid, except in one larva that resembled the maternal type so closely that it is probably not a hybrid at all, but a pure type due to contamination, or to parthenogenetic development. Possibly it is a triploid.

Among the numerous crosses that Tennent has made with Caribbean and West Atlantic types of the sea-urchin, several are here described as the most interesting. When the eggs of Toxopneustes are fertilized with the sperm of Hipponoë, the cleavage takes place in the tempo characteristic of the egg. The hybrid pluteus that results shows "a pronounced Hipponoë dominance" as expressed in the great number of fenestrated anal arm rods, in the number of multiple rods, and in the general form of the body skeleton. These relations are shown by comparing the pluteus of Toxopneustes (Fig. 256*a*) and that of Hipponoë (Fig. 256*b*) with that of the hybrid (Fig. 257*a-b* and *c-d*). The extent of the paternal influence through the sperm is shown in detail in the following table: (Table XXVIII).

TABLE XXVIII

Summary of Results of Cross-Fertilization in Ordinary Sea Water

[Number of plutei studied, 50. Temperature of water, 28.5° C.]

Year	Cross	Plutei with Lattice Structure	Anal Arm Rods with Lattice Structure	Arms More than One Rod	Perfect Hipponoë Rods	Perfect Toxopneustes Rods	Perfect Toxopneustes Plutei	Perfect Hipponoë Plutei	Basket
1909	Hip. ♂ / Tox. ♀	33	60	39	14	1	0	5	10
1910	Hip. ♂ / Tox. ♀	24	32	60	0	8	0	0	25

This table shows that in 66 per cent of the plutei, the arms had a latticed structure; there were no perfect Toxopneustes plutei, and 5 per cent pure type Hipponoë plutei occurred. In the reciprocal cross, Hipponoë eggs and Toxopneustes sperm (Fig. 257*a*, *b*), the dominance of the Hipponoë elements again become apparent in the hybrid pluteus. The details of the characters of the hybrids is given in the following table:

TABLE XXIX

SUMMARY OF RESULTS OF CROSS-FERTILIZATION IN ORDINARY SEA-WATER

[Number of plutei studied, 50. Temperature of water, 28.5° C.]

Cross	Plutei with Lattice Structure	Anal Arm Rods with Lattice Structure	Arms More than One Rod	Perfect Hipponoë Rods	Perfect Toxopneustes Rods	Perfect Toxopneustes Plutei	Perfect Hipponoë Plutei
Tox. ♂ / Hip. ♀	37	58	40	30	2	0	12

This analysis shows that 74 per cent of the plutei have anal arms with rods having a fenestrated structure.

In another cross with Toxopneustes eggs and Mellita sperm, the hybrids were of the "mixed type" resembling in general more the maternal type in form of body, but having without exception multiple rods in the anal arms. A few of the hybrid plutei resembled the pure maternal form, although their hybrid origin was also evident.

When the eggs of Toxopneustes are fertilized by sperm of Moira, a spatangoid, the greater number of the hybrids were of the "intermediate maternal type," having multiple rods in the anal arms. A very small number of pure Toxopneustes plutei (1 per cent) were also found, suggesting chance fertilization by Toxopneustes sperm, but this possibility is, Tennent thinks, excluded. It is not improbable that these exceptions may be due to the exclusion of the sperm-nucleus, and if so the plutei are haploid or diploid parthenogenetic.

The reciprocal cross, Moira eggs by Toxopneustes sperm, gave also an intermediate type of pluteus. There was no trace of the maternal posterior unpaired spine, although the larvae lived seven days. Its absence may be ascribed to the paternal influence.

The eggs of the primitive form, Cidaris tribuloides, are easily fertilized by the sperm of Toxopneustes (Lytechinus) variagatus (Tennant '14). More than 90 per cent of the eggs are fertilized. The development up to the time of gastrulation is similar to that of Cidaris. The hybrid begins to gastrulate, but does not pass beyond the gastrula stage. The difference in the time and manner of mesenchyme formation of the parent types and those of the

hybrids will be described later. The pluteus stage of Cidaris is drawn in Fig. 258*a*, and a corresponding stage of Toxopneustes in Fig. 258*b*.

Tennent also obtained segmentation stages of the egg of

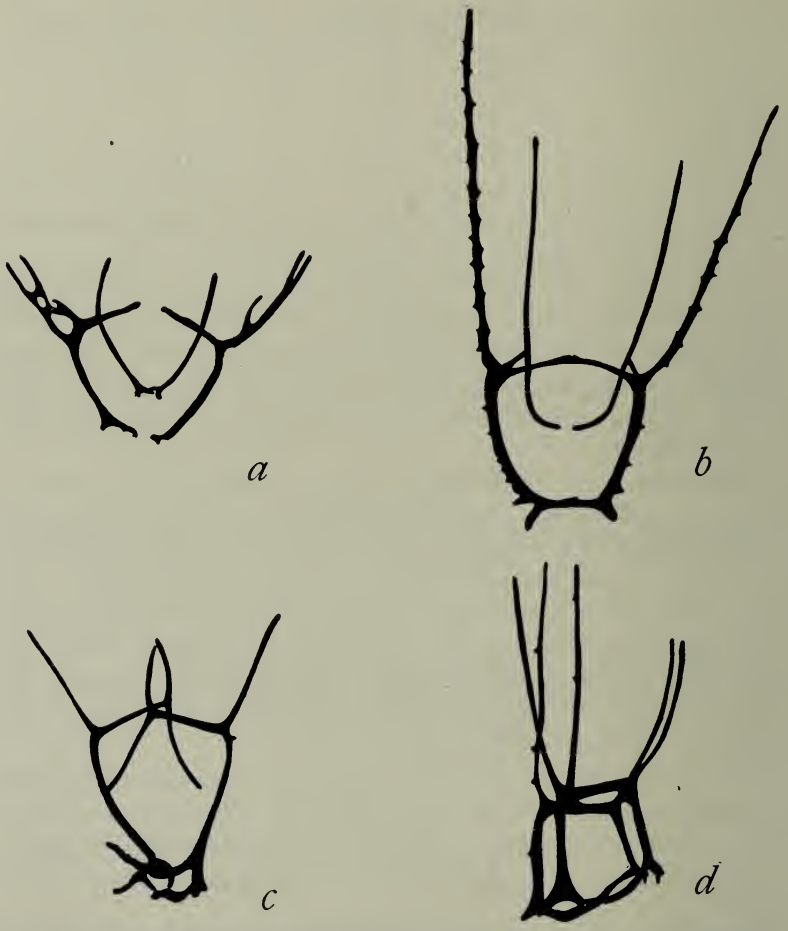


Fig. 257.—*a*, *b*, skeletons of plutei of Hipponoë ♀ by Toxopneustes ♂; *c*, *d*, reciprocals of last, viz., Toxopneustes ♀ by Hipponoë ♂. (After Tennent.)

Toxopneustes fertilized by the sperm of a holothurian. Irregular cleavage took place, but no later stages developed.

The hybrids produced in the experiments of Shearer, de Morgan and Fuchs ('11) were carried much farther than the hybrids

reared by earlier workers. Some of them were carried through the pluteus stages into the echinus stage. Three types of sea-urchin, found at Plymouth, England, were crossed, namely, Echinus acutus, Echinus esculentus, and Echinus miliaris. The first two are closely similar forms, yet are recognized as good species, and have, according to Doncaster and Gray, different chromosome numbers (38 and 36). Shearer, de Morgan, and Fuchs point out that the pluteus skeleton of the species is subject to so much variation even in the pure types, that it is an unsafe criterion on which to base conclusions concerning the resemblance of the hybrid characters to those of its parents. While this contention is generally recognized to-day for other types also,

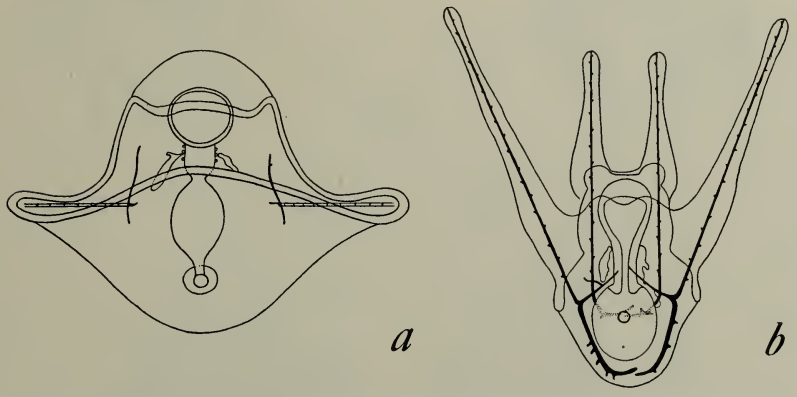


FIG. 258.—*a*, pluteus of Cidaris; *b*, pluteus of Toxopneustes. (After Tennent.)

nevertheless, the evidence from this source can be used, provided a wide enough range of types is considered, and if other conditions, both internal and external are kept under control. The two or three older characters used by Shearer, de Morgan, and Fuchs, while more constant in the parental types, are also variable to some extent in the hybrid, and this variability has also made comparisons difficult when the hybrids are contrasted with the parental types.

A fully formed pluteus of E. esculentus is shown in Fig. 259 (above to the right). Two preoral arms have grown out, and two partially ciliated and much pigmented rings have appeared, the anterior and posterior epaulettes. On the left side of the figure, the rudiment of the young echinus is seen inside the

pluteus. A pair of pedicellariae on the left side and one posterior pedicellaria are present (l. ped, and p. ped. Fig. 259, above to the right).

A corresponding stage of E. acutus is shown in Fig. 260 (above to the right). The resemblance of the plutei of the two

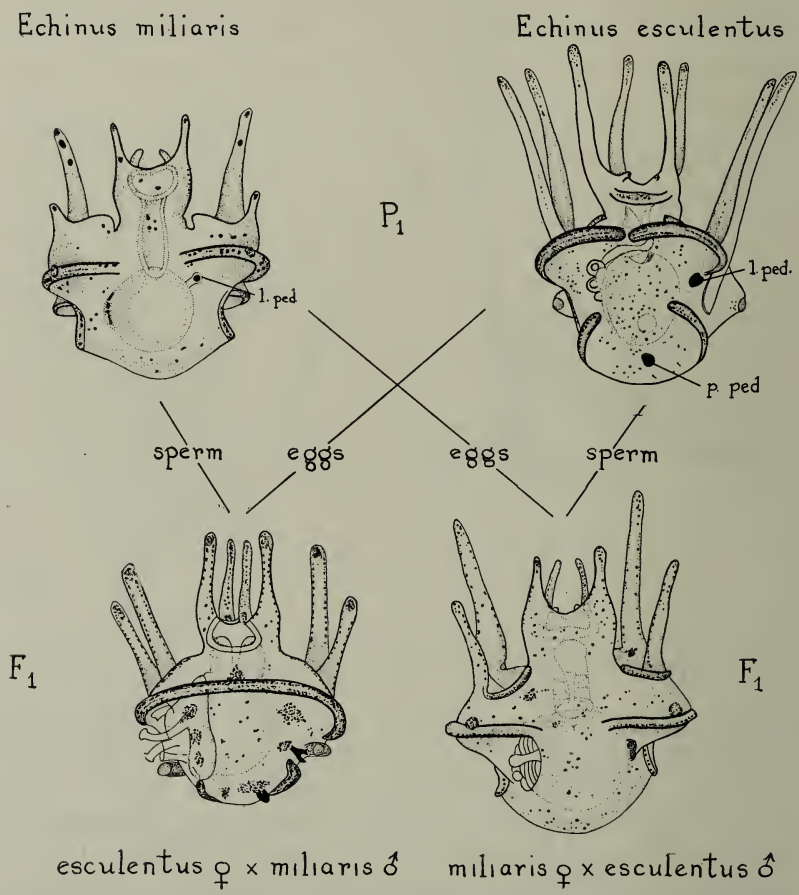


Fig. 259.—Plutei of Echinus miliaris and E. esculentus (above), and of the two reciprocal hybrids (below). (After Shearer, de Morgan, and Fuchs.)

species is seen to be very close; the most pronounced difference is in the skeleton of early larvae. In acutus the aboral ends of the body rods are more robust and more spinous, and not so much bent toward the middle line.

The eggs of E. miliaris are smaller than those of the other two

species. The arms of the pluteus are shorter and the aboral end more pointed. There is less pigment than in E. esculentus. A 20 day pluteus is shown in Fig. 259 (above to the left). The anterior epaulettes are present. There is no trace of posterior epaulettes. In the anterior epaulettes there are four masses of green pigment. This pigment is absent in the other species, and this difference is used as one of the more definite diagnostic differences between

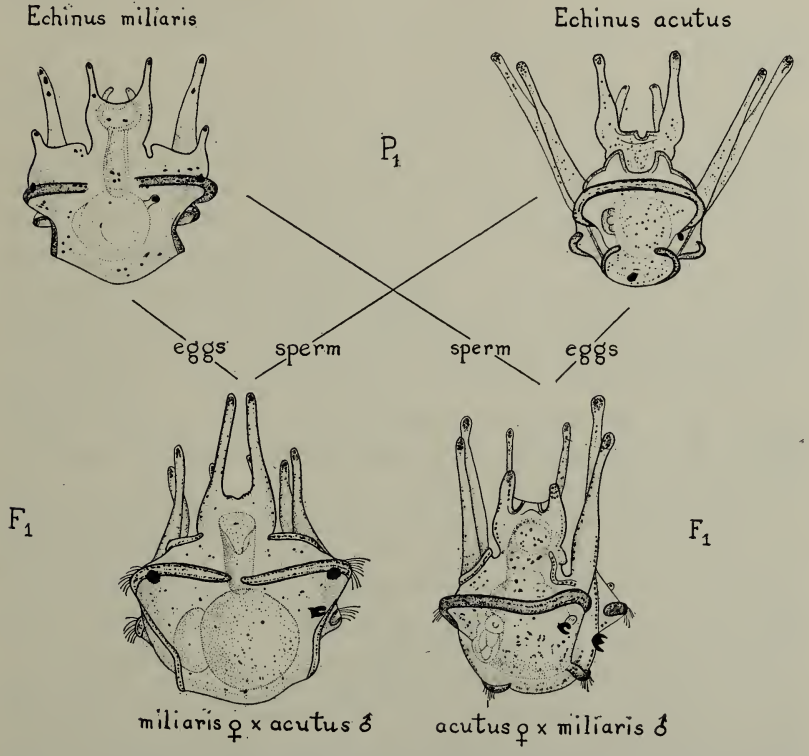


Fig. 260.—Plutei of Echinus miliaris and E. acutus (above), and of the reciprocal hydrids (below). (After Shearer, de Morgan, and Fuchs.)

this and the other two types. Furthermore, while the pluteus of E. miliaris never develops a posterior pedicillaria, this structure is typically present in the other two forms, although it may sometimes not appear in them.

Two series of experiments were made in different years. The results were somewhat contradictory. The two series will be given separately.

The first cross, E. esculentus eggs by E. miliaris sperm, gave the pluteus drawn in Fig. 259 (below to the left). Both the posterior and anterior (maternal) epaulettes are present, as well as the posterior pedicellariae (maternal). No green masses were present. The reciprocal cross, eggs of E. miliaris by sperm of esculentus gave the pluteus drawn in Fig. 259 (below to the right). The posterior epaulettes (paternal) did not appear, but the green pigment (maternal) was present. In these two crosses then, the older hybrid larvae showed maternal characters.

When the eggs of E. acutus were fertilized with the sperm of E. miliaris they gave the following results (Fig. 260 below to the right). Anterior and posterior (maternal) epaulettes appeared, as well as a posterior pedicellaria (maternal), but no green pigment (paternal). The reciprocal cross, eggs of E. miliaris and sperm of E. acutus gave the pluteus drawn in Fig. 260 (below to the left). The anterior epaulettes with green pigment (maternal) were present. The posterior epaulettes never developed, nor were the posterior pedicellariae present.

In this cross the results were the same as before. The larvae showed only the maternal characters in which the two species differ. In other words, the characters in question came from the egg and not from the sperm.

A few more general features may be noted. The hybrids developed more slowly than the pure type larvae. The posterior epaulettes of the hybrid never reached the same degree of development as in the pure form, and this applies in like degree to the skeleton, pigment and pedicellariae.

As stated above somewhat different results were obtained in a later (1912) series of crosses. These may now be described. The cross between eggs of E. esculentus and sperm of E. miliaris gave the same results as before, i.e., the later hybrids showed maternal characteristics except in one cross in which four individuals developed both the posterior epaulettes, eight had an epaulette only on one side, but eleven had none. No green pigment was present.

When the eggs of E. miliaris were fertilized by the sperm of E. esculentus not more than 20 per cent of the eggs developed, while in former years 80 per cent developed. "With two exceptions all the cultures . . . gave larvae with paternal inheritance,

which was exactly the opposite of the condition in 1910-1911. In general form the larvae were of the E. esculentus type. They developed the posterior ciliated epaulettes and lacked the green pigment." One cross was exceptional. All the larvae had the paternal absence of pigment, but they differed amongst themselves in respect to the posterior epaulettes; eighteen had both epaulettes, five had one on one side only, and nine had neither posterior epaulettes.

When the eggs of E. miliaris were fertilized by the sperm of E. acutus all the cultures without exception gave purely paternal plutei—the reverse of the earlier experiments.

When reciprocally the eggs of E. acutus were fertilized by the sperm of E. miliaris the larvae were maternal, as previously, with one exception. Here all the larvae had the maternal absence of green pigment, but some had both posterior epaulettes, some had one and others had none.

Excluding the exceptional cases for the moment, two of the crosses gave the maternal inheritance of previous years, while the other reversed the result of former years, giving paternal inheritance.

It is interesting to note in this connection that Debaisieux working in London in 1912 on the same material from Plymouth obtained exactly the same results as those of Shearer, de Morgan and Fuchs in 1912, except for a few variations observed by the latter workers.

Just after metamorphosis the young sea-urchins of E. miliaris and E. esculentus show one characteristic difference first observed by MacBride. After this time no structural difference is noticeable until the sea-urchin has grown to considerable size. The difference referred to concerns the tube-feet. At metamorphosis E. esculentus has one terminal tube-foot in each radius, while E. miliaris has in addition five pairs of lateral tube-feet. In the hybrids, however, there was found so much variation in the same cross that no definite statement concerning maternal or paternal inheritance was possible.

The contradictions between the results of earlier and later crosses are discussed by the authors, but no definite conclusions are reached. The probability of their being due to environmental differences can better be appreciated after other evidence has been given, especially that of Tennent and of Koehler (see below).

Furthermore, the cytological findings of Doncaster and Gray based on the same material will have to be considered, especially in connection with the observations of Baltzer.

Hybrid urchins one year old derived from eggs of E. miliaris and sperm of E. acutus have been reported on by Mortensen, who states that the hybrids resemble the maternal parent more than the paternal in certain details of color and structure, but in other characters they are partly paternal and partly intermediate.

Baltzer ('10) has described hybrids from four combinations of sea-urchins and since some of these have an important relation

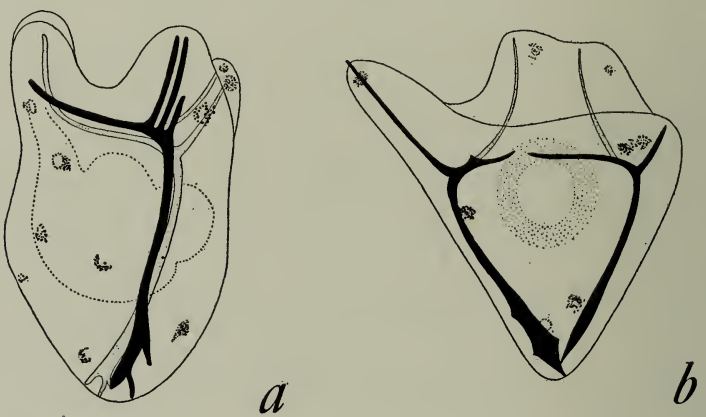


FIG. 261.—*a*, hybrid pluteus, Sphaerechinus ♀ by Strongylocentrotus ♂; *b*, reciprocal of last, viz., Sphaerechinus ♂ by Strongylocentrotus ♀. (After Baltzer.)

to the behavior of the chromosomes in the crosses they may be described here. The pluteus of Sphaerechinus is drawn in Fig. 101*c*. That of Strongylocentrotus is known from descriptions of Vernon ('98), Morgan ('94), Steinbrück ('02), Baltzer ('09). It is shown in Fig. 248*a*. The hybrid from the eggs of Sphaerechinus and the sperm of Strongylocentrotus is intermediate (Fig. 261*b*). The reciprocal cross produces a hybrid whose skeleton in several respects at least, is so similar to that of Strongylocentrotus (maternal) that no certain differences have been found between them (Fig. 261*b*).

Crosses between the spatangoid "sand dollar," Echinarachnius parma (♀) and the sea-urchin Arbacia punctulata (♂) have been made by Matsui ('24). Without the addition of sodium

hydroxide, not more than 5 per cent of the eggs are fertilized; with it more than 30 per cent of the eggs may be fertilized. The hybrid pluteus is said to be like that of the maternal species. In the absence of the reciprocal cross it cannot be certain whether the resemblance is one of dominance or whether the inheritance is maternal, but in the light of other crosses it seems more probable that this is another case of dominance of the more striking characters of one species. Arbacia has 38 chromosomes (n-19) [1] and Echinarachnius 52 (n-26). There are 45 chromosomes in the cross-fertilized eggs. After the second division the number is reduced to 40 or 41, due probably to the elimination of some of the chromosomes.

Dominant and Recessive Larval Characters

A certain vagueness is attached to descriptions of the characters of the hybrid larval stages of sea-urchins when they are compared with those of the two parent larval types. An attempt to avoid this has been made by most of the later writers on the subject, by comparing separate parts such as the skeleton, the arms, or the pigments of the parental with those of larval hybrid types, but even then the comparisons are often a matter of opinion. A more definite attempt towards clarifying the comparisons was made by Loeb, King, and Moore ('12) who introduced the Mendelian terms dominant and recessive—terms that had come to have a special meaning to students of heredity. It was assumed that the single characters of the hybrid would be expected to be either dominant or recessive, i.e., it was implied that the individual characters of the two parental types would be, in their relation to each other, either dominant or recessive in the hybrid. But this relation is exceptional rather than the rule, even in adult characters that follow Mendel's law; for, in many cases, the hybrid character is intermediate, i.e., neither dominant nor recessive. In the second place the Mendelian estimate of what is dominant and what recessive and what character or part of a character is an allelomorph of another one is based, in large part, on the recombinations that appear in the second generation. Since a second generation has not been reared in any of these larvae, the essential criterion is absent. There

[1] E. B. Wilson recorded 36 to 38 for Arbacia as have some other students.

are even more serious difficulties in the way of applying these terms to young larvae a few days old. It has been shown that there is at work here an influence that is not present when adult characters are contrasted. The sperm nucleus does not affect at once, or only to a small degree, the foreign protoplasm in which it finds itself; hence there is often an egg-influence (cytoplasmic) that may have a significant bearing on the results. Reciprocal crosses, to be sure, help to show how far this influence is determinative on the characters in question, but even such comparisons do not remove all the difficulties. Moreover, the terms in question have been used when the reciprocal cross was not even known.

Again, the more different the parent species, the greater are the difficulties of finding out what characters form Mendelian pairs. In fact, the typical Mendelian situation is one in which the two parent forms differ in one character and its allelomorph, *and are alike in all other characters.* In the case of most of the sea-urchin crosses, the parent species differ from each other in a large number of characters.

There may be also a question as to whether the absence of a character in a hybrid is due to its recessiveness, *sensu strictu*, or to an abnormal conditon of the hybrid larva that so seriously affects it that certain characters can not fully develop.

Influence of Environmental Factors on Echinoderm Larvae, Pure and Hybrid

The great amount of variability shown by the plutei of sea-urchins has led to several attempts to find out how far the variability is due to external and how far to internal factors.

Vernon's experiments ('95) with the larvae of Strongylocentrotus lividus were undertaken to study the effects of temperature, of concentration of sea water, of light, and of chemical agents on the development of the pluteus of sea-urchins. He found that when the eggs at the time of fertilization were placed in sea water at 8 degrees or at 25 degrees C. for a minute or for an hour, the resulting plutei (eight days later) were 4.4 per cent smaller than those fertilized between 17 and 25 degrees C.

The normal breeding season for Strongylocentrotus is from December to March. Larvae in August were found to be 20 per cent smaller than those in April or in October, due probably to immaturity.

The addition of 50 cc. of distilled water to a liter of sea water gives larvae 15.6 per cent smaller than those in pure sea water. The addition of 25 cc. distilled water gives larvae 9.5 per cent larger than in sea water.

Vernon found differences of size in larvae grown in darkness and in light of different colors; but here the effects are probably indirectly due to the light affecting the growth of bacteria in the water, although Vernon does not think the results are due to such causes.

The plutei of Strongylocentrotus were largest at a temperature of 23.7 degrees C.; Sphaerechinus at 15.9 degrees C., and Echinus at 20.4 degrees C. These temperatures represent the optimum for size at least for these species.

Herbst ('06) found that with increase in temperature, the plutei of Echinus showed an increase in the number of the multiple anal rods; they were smaller at 24-25 degrees than at lower temperatures, and showed sometimes the beginning of a lattice work. In Sphaerechinus, he found, that, with increase in temperature, the number of rods in the anal arms increased from three to six, at least in the basal portion of the arms. The length of the body at 13 degrees is greater than at 25 degrees and the rods in the arms are larger in the cooler temperature.

The seasonal variability in the *hybrid* plutei from a large number of crosses has been studied by Vernon ('95). In the most favorable combinations, namely Echinus-Arbacia, Echinus-Strongylocentrotus, Sphaerechinus-Echinus, and Sphaerechinus-Strongylocentrotus he found that the summer hybrid larvae were more matroclinous, while the autumn and winter hybrid larvae were more patroclinous. He drew the conclusion, which has been, in part, but in a different sense, also maintained by the more detailed work of Koehler ('15), that these differences in type are due to the relative maturity of the eggs and sperm. Later Doncaster ('03) attempted to show that the results of the kind observed by Vernon were due to temperature but, as will be shown below, it is doubtful if this is the real explanation, even although some effect may still be ascribed to temperature. Herbst, also, thinks that Vernon's seasonal differences were due to temperature, but only partly so. He does not ascribe much influence to the ripeness of the germ-cells.

Herbst ('06) studied in much detail the character of the

hybrid plutei from Sphaerechinus ♀ by Echinus ♂ and of Sphaerechinus ♀ by Strongylocentrotus ♂ at different temperatures (11-19 degrees and 24-27 degrees C.). More latticed structures occurred between 23-29 degrees than below 20 degrees C. At the higher temperature there were more short skeletal rods. The increase in the latticed structure in the hybrid at higher temperatures, when Sphaerechinus eggs were used, may not have been due to a maternal influence but directly to the temperature, as Tennent points out, because the same effect is seen in the pure larva of Sphaerechinus. Similar relations may account for other so-called maternal effects in the hybrid. It was also found that to produce certain temperature effects on the skeleton it is necessary to keep the embryos at that temperature up to or even throughout the gastrula stages, i.e., to a time when the foundations of the skeletal forming cells are laid down.

A very extensive study of the causes of variability of the plutei of two species of sea-urchins and of the hybrid resulting from a cross between them has recently been carried out by Koehler ('15). The experiments were made at Naples and extended over an entire year; they were confined, almost exclusively, to measurements of the skeleton. Large numbers of plutei were measured and biometrical methods utilized in interpreting the results. Careful controls at every point give the results special value. When a cross was to be made, normal control cultures were also made, and the plutei reared under the same conditions as the hybrids. Comparisons were made only with these controls. The cross was always made the same way, viz., Sphaerechinus female by Strongylocentrotus male. Koehler finds that while some of the single characters of the pluteus skeletons of the two species show constant differences, others may slightly overlap, i.e., show transgressive variability. The skeleton of the hybrid is in many respects "intermediate." Very rarely is the hybrid, in the sum total of its characters, purely patroclinous or matroclinous. On the other hand, single characters are more often purely patroclinous or purely matroclinous. If all the characters are taken into account their mean value is more or less patroclinous.

It makes no difference if the eggs, before or during or just after fertilization, are kept in sea water containing different amounts of oxygen, or salt, or different degrees of alkalinity

—the mean measurements are the same. Temperature has a different effect. About half of the cultures are not affected, but the others are affected. These are sometimes made more patroclinous, sometimes more matroclinous, depending on the chance selection of the parents. When so changed by temperature, the effects are due to strengthening or weakening the maternal or paternal influence in exactly the same way as the pure plutei are affected, causing their particular characters to be strengthened or weakened.

The most novel part of Koehler's experiments relate to eggs taken from different parts of the ovary. Those nearest the exit pores are the oldest—ripest—those in the middle regions are not quite so ripe, while those in the recesses of the gonads are the youngest, although all of these have given off the polar bodies. In some animals, the oldest and youngest eggs show the species characters equally developed; in other individuals the oldest, in others the youngest eggs, develop the species characters most strongly. When plutei from the oldest and youngest eggs show a marked difference, the intermediate eggs show an intermediate condition. When both develop in the same way the intermediates are like both.

Seasonal dimorphism was not found. Recorded results of this kind are believed by Koehler to be due to unhealthy larvae being used for comparison.

Koehler draws the following conclusions from his evidence. Each gamete (egg and sperm) undergoes a periodic change in the strength of its hereditary tendencies from the time of its maturation divisions to its degeneration. The strength of the heredity influence rises gradually from a lesser influence at first to a maximum and then decreases. The hereditary characteristics of the larvae are the result of the antagonistic influences of the egg and sperm as determined by their age. He also thinks that this may not be the only cause of the changes in question, for different hereditary factors may be present in different individuals, and the influence of the environment on the germ-cells while inside the parents may also have an influence in so far as it affects their ripening. This point is left undetermined. These statements apply both to the pure forms and to the hybrids.

The attempt to correlate the characteristic structures of the

species with the age of the egg and sperm, and the preponderating effects of this influence on the cross raises several perplexing questions. The cytoplasm has always been under the influence of the chromosomes, and it is difficult to imagine just why the relatively short period after extrusion of the polar bodies, when only half of the chromosomes are present, should have a preponderating influence. If the same kind of influence affects the sperm as Koehler thinks his evidence shows, then, since only the nucleus of the sperm enters the egg the influence must be referred to a change in the chromosomes, rather than to an imagined effect of the chromosomes on the cytoplasm as in the case of the egg.

The clearest cut cases of the influence of the environment on the characters of the hybrid pluteus are those found by Tennent ('11). He studied the hybrids between Hipponoë and Toxopneustes (Fig. 256 and 257). In general "a pronounced Hipponoë dominance is shown" as expressed in the great number of fenestrated anal arm rods, in the number of multiple rods, and in the general form of the body skeleton. The presence of more than one anal rod and of a basket-like structure in the posterior region of the body are interpreted to mean a Hipponoë influence. By increasing the alkalinity of the sea water in which the young larvae were reared (adding 20 drops of N/10 NaOH to 400 cc. of sea water) the hybrid plutei obtained were still more like the Hipponoë type (Fig. 256*c*, *d*).

The reverse effects were obtained by decreasing the alkalinity. This was accomplished by the addition of a small amount of acetic or hydrochloric acid to the sea water. The hybrid plutei were then more like the Toxopneustes types as shown in Fig. 257*c*, *d*.

In interpreting these results one should have, for comparison, control experiments made at the same time in which the influence of alkalinity and acidity were tested for the pure plutei. If it should be found that changes took place in the same direction as those shown by the hybrid the results might then be interpreted to mean that the direct action of the agents affects the pure pluteus in the same way as it affects the hybrid. Nevertheless, it might still, perhaps, be said that the "dominance" is affected by the external medium, for the dominance even of Mendelian characters may depend on environmental conditions.

The Cytoplasmic Influence on the Characters of the Embryo and Larva

Each egg comes to maturity in the ovary in the presence of a full (diploid) set of chromosomes. Half of these chromosomes are lost in the polar body in the maturation process. The sperm-nucleus makes good again the full number. How soon the paternal chromosome affects the cytoplasm can be determined only in cases where the sperm comes from another type (species or variety) in which the early stages are different from the type to which the egg belongs. Several cases of this sort have been studied, especially in echinoderms and fishes.

The cleavage pattern of the eggs of sea-urchins is much the same in different species, and it has so far not been used for diagnostic work of this kind. The tempo of the cleavage, however, may be different in different species of sea-urchins, and has been utilized by Driesch, who found that the cross-fertilized egg follows the rate characteristic of the egg, and is not influenced by the sperm.

The size of the blastula, which is largely dependent on the size of the egg, is also said to be the same as that of the egg parent; but inasmuch as the hybrid blastulae are often delayed somewhat in their development, it is difficult to tell whether the size, if less than that of the egg species (when this has the larger egg of the two kinds under comparison), is due to the influence of the sperm, or due to slowness or defects in development.

The same question arises concerning the shape and size of the young triangular larvae of the sea-urchin (the stage at which the spicules first appear). Boveri thought that the paternal influence is shown at this time, both in the size and the shape of the larvae, when Sphaerechinus eggs were fertilized with Strongylocentrotus sperm. But Driesch's results, with apparently better material, appear to show that the hybrid larvae at this time can not be distinguished from larvae of the maternal type. Nevertheless, since the general shape of the pluteus stage, which immediately follows the triangular stage, shows in many cases the influence of the sperm, it is not improbable that Boveri is to some extent right in his contention.

The mesenchyme cells of the sea-urchin larvae, that are given off from one end of the blastula, at the time when the blastula

is about to gastrulate, are definite in number, and can be accurately counted. Driesch's results show very plainly that their number agrees with the number characteristic of the egg-species. His results are as follows: Sphaerechinus has an average of 38 mesenchyme cells; Echinus 57; the hybrid (Sphaer. eggs and Echinus sperm) has 37.2. Strongylocentrotus has on an average 47.6 mesenchyme cells. The hybrid (Sphaer. eggs and Str. sperm) has 37.2. Echinus has 57 mesenchyme cells. The hybrid (Sphaer. egg by Echinus sperm) has 37.2. In every case the agreement between the number of mesenchyme cells of the egg-species and that of the hybrid is extremely close. The sperm produces no effect on the number of mesenchyme cells that appear in the hybrid.

The pigment of the larva is dependent in part on that present in the egg, and in part on the development of new pigment in the pluteus, especially in certain mesenchyme cells. Boveri showed in one species of Strongylocentrotus from Villafranca that some of the pigment in the ring goes over into the chromatophores (secondary mesenchyme) of the pluteus. In the Neapolitan variety of this same species less pigment is present in the ring, but the eggs of one individual may be different from those of another. Boveri found that the amount present in the mesenchyme corresponds to the amount characteristic of the eggs of the individual used. In addition, he observed that some of the eggs, that were entirely without pigment, developed pigmented chromatophores, showing their power to produce pigment later.

Boveri also found that when the eggs of an individual of Echinus are fertilized by sperm of other individuals of the same species, the amount of pigment that develops in the plutei shows in each culture characteristic differences, and since the eggs are the same throughout, the difference must be due to the influence of the sperm. This pigment is, however, the later pigment that develops in the pluteus, over and beyond that already present in the egg.

Driesch found that when heavily pigmented eggs such as those of Arbacia are fertilized by sperm from a species with an unpigmented egg, the color of the mesenchyme of the young embryo is the same as that of the maternal species. And conversely, the sperm of Arbacia does not affect the general coloration of the hybrid embryo from eggs that have no pigment. In

other words, the early pigmentation, or its absence, is referable directly to the egg, and shows no influence from the sperm-nucleus of the other species.

The most convincing evidence as to the time at which the sperm of the sea-urchin's egg may begin to affect the developmental process comes from Tennent's very thorough work on the cross between the egg of Cidaris and the sperm of Lytechinus (Toxopneustes). There is a very striking difference in the rate of development between these two sea-urchins, as the following table shows:

TABLE XXX

Cidaris	Hours	Lytechinus	Hours
Blastulae (swimming)	16 to 18	Blastulae (swimming)	5.5
Gastrulae (beginning)	20 to 23	Mesenchyme	8
Mesenchyme	23 to 26	Gastrulae (beginning)	9
Chromatophores	44	Chromatophores	15 to 16
Skeleton (beginning)	72 to 73	Skeleton (beginning)	15 to 16
Pluteus	120	Pluteus	24

Gastrulation in Cidaris begins at about the twentieth hour after fertilization, that in Lytechinus at the ninth hour. The mesenchyme cells begin to migrate into the interior of the blastula cavity at about the twenty-third hour in Cidaris, and at the eighth hour in Lytechinus. In Cidaris the mesenchyme cells develop from the inner end of the archenteron; in Lytechinus these cells begin to migrate before the gastrulation process has set in.

The cross-fertilized egg (Cidaris egg, Lytechinus sperm), divides in a typical way, and no effect of the sperm is observed before the beginning of gastrulation. The rate of the cleavage is not hastened, and the blastula has the appearance of that of Cidaris. The mesenchyme cells arise from the sides and around the base of the archenteron in the hybrid, and not from its inner end as in the normal Cidaris blastula. Their appearance "seemed slightly hastened although not sufficiently to warrant a general conclusion." But as to the place of origin of the mesenchyme there could be no question, the effect of the Lytechinus sperm was apparent, for in the hybrid, as stated above, the mesenchyme cells arise from the wall of the archenteron just as the latter

begins to invaginate. In this respect the formation of the mesenchyme is intermediate between that of the two species.[2]

The Chromosomes of Hybrid Sea-Urchins

Baltzer's discovery ('09), that in certain hybrid combinations some of the paternal chromosomes are eliminated at the first cleavage of the egg, has been used to explain the matroclinous character shown by some hybrid plutei. This interpretation is further supported by the conditions in the reciprocal cross, where none of the chromosomes are eliminated, and the hybrid pluteus is intermediate between the parental types of plutei. The following combinations are those that have so far been made, and illustrate the main point at issue, namely, that while the elimination of the chromosomes is fairly well established in a few cases, there still remains much to be done along these lines. The difficulty in getting the necessary information is due to the irregularities in the elimination of the chromosomes, since no two eggs give exactly the same chromosome picture. Hence the complete story has to be patched up from individual cases, each taken at one and only one stage in its development. There is often a difficult question of correct seriation.

Strongylocentrotus by *Sphaerechinus.* When the eggs of Strongylocentrotus are fertilized by sperm of Sphaerechinus, the blastulae are abnormal. They are turbid, and often filled with irregular masses of cells. Few reach the pluteus stage. The skeleton of those that reach this stage resembles that of Strongylocentrotus (matroclinous). The form of the body is very variable, owing probably to the pathological condition of earlier stages. Irregularities occur in the first mitosis, which is delayed. Up to the time of division of the chromosomes, the process, although delayed, is typical; but some of the divided chromosomes fail to separate (Figs. 262*b*), and while most of them reach the poles, others remain scattered in the middle of the spindle. These are sometimes caught at the cell-wall when cleavage takes place; others may, however, pass into the reconstructed nuclei. At the next division, something like the same state of affairs is seen—

[2] The hybrid does not develop much beyond the gastrula stage. There is evidence of irregular divisions in some of the chromosomes, and probably some elimination at the first cleavage.

chromosomes lagging or clumped in the middle of the spindle. Some of these are later lost. Counts of the chromosomes, after the first cleavage, give about 22 elements. About the same number, or a few less, are found in the 2- and 4-cell stages. In older stages about 21 are found. Thus instead of the full complement of 38, about 16 or 17 are absent. There can be little doubt that these lost chromosomes are paternal, i.e., of the 20 paternal elements only four go through a normal division. That the loss is from the paternal set was shown by Baltzer in the following

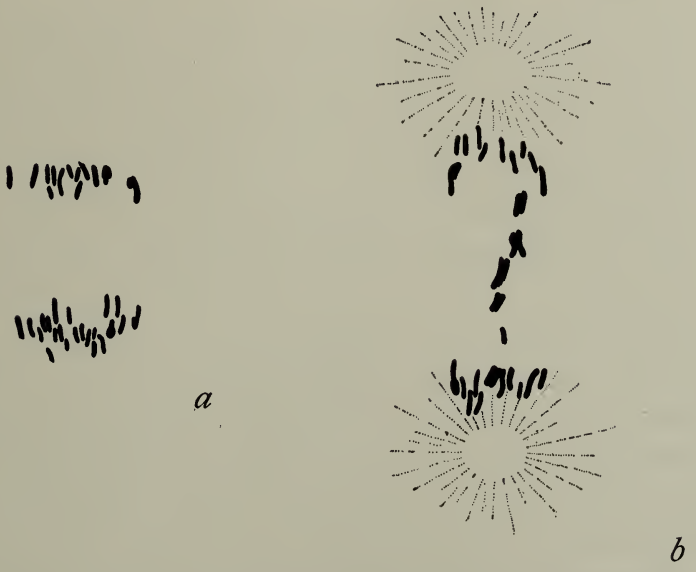


Fig. 262.—*a*, side view of segmentation spindle of hybrid between Sphaerechinus ♀ by Strongylocentrotus ♂; *b*, lagging chromosomes on segmentation spindle of hybrid between Strongylocentrotus ♀ by Sphaerechinus ♂. (After Baltzer.)

way. When non-nucleated fragments of Strongylocentrotus eggs were fertilized by Sphaerechinus sperm, the haploid spindle shows only four chromosomes that divide regularly—the remaining sixteen are abnormal.

The reciprocal cross, Sphaerechinus egg by Strongylocentrotus sperm, is more successful in that fewer eggs develop irregularly. The skeleton of the pluteus is intermediate (or mixed) as seen by comparing Fig. 101*c*, *f*, for Sphaerechinus with that of Fig. 261*a*, *b*, for the hybrids. When the changes that take

place in the nuclei are followed, it is found that the two pronuclei fuse completely as in normal fertilization. The first spindle shows both characteristic forms of chromosomes (Fig. 262*a*). All the chromosomes are present in the dividing spindle. These results are in accord with the intermediate character of the hybrids that contain the full number of chromosomes. It may seem probable, therefore, that in the reverse combination, where most of the paternal chromosomes are lost, the matroclinous character of the hybrid is due to the almost complete absence of the paternal set of chromosomes.

Sphaerechinus by *Echinus*. The skeleton of the hybrid pluteus from Sphaerechinus female by Echinus male is intermediate in character, i.e., it shows characters peculiar to both the paternal and maternal types. No elimination of chromosomes takes place. The reciprocal cross is difficult to make. The 20-hour embryos were sickly and died as blastulae, or as young gastrulae. In the first cleavage, elimination of chromosomes takes place. The full number in the segmentation spindle should be 38. Only about 22 go through the normal divisions, i.e., about 16 chromosomes are eliminated.

Arbacia by *Sphaerechinus*. The cross with Arbacia egg and Sphaerechinus sperm gives sickly blastulae that die; rarely are plutei formed, and, then, they are too abnormal to show the characters of the skeleton, according to Baltzer, but Doncaster records that "these plutei closely resembled pure-bred Arbacia larvae." Fischel noted, however, in the apical rods, an influence from the paternal parent, but in other respects a maternal influence was shown. Elimination of chromosomes takes place, both in the first and second divisions. In the second division about 22 chromosomes divide normally. Spindles in the 4- and 8-cell stages gave 22 chromosomes. Since 40 chromosomes is the full number, there must be an elimination of 18 elements. From the shape of the chromosomes that divide normally, Baltzer concludes that those that are eliminated belong entirely to Sphaerechinus. None of the maternal chromosomes are eliminated.

In the reciprocal cross, Arbacia sperm and Sphaerechinus eggs, no elimination takes place in the first division of the egg, yet the plutei are matroclinous.

Strongylocentrotus by *Arbacia*. When Strongylocentrotus eggs are fertilized by Arbacia sperm—a combination in which

nearly all the eggs segment—the blastulae are often sickly with cell accumulations in the interior. Many of these blastulae develop no further. A few form plutei. The skeleton of the pluteus is purely matroclinous. Of the 60 hybrid plutei studied by Baltzer, 30 had purely matroclinous skeletons; the rest showed some type of abnormality. As already stated, the full number of chromosomes appears in the first division, viz. 38. The same number approximately is found in the 8-, 16-, 32-cell stages. The size of the nuclei in the hybrid gastrula stage is a little smaller than that of the pure Strongylocentrotus gastrula stage. This difference is even more marked in the pluteus stage, and cannot be ascribed to the late development of the hybrid. The average diameter of nuclei of the hybrid is 21.2 and of the pure form 36.4. It is to be noted that the nuclei of the pure Arbacia pluteus are a little smaller than those of the pure Strongylocentrotus pluteus. The nuclei of the hybrid might, therefore, be expected to stand midway between; but even when allowance is made for this expectation the nuclei of the hybrid are still much below the intermediate size.

In two cases the chromosomes of a hybrid pluteus were counted and were found to be only 17 in one and 18 in the other case—a reduction to about half the number (the full number would be 38 for this combination). Elimination has, therefore, in all probability taken place in the gastrula or later stages and this change may appear to coincide with the transition from blastula to gastrula when the embryos suddenly appear to become sickly. An examination of the nuclei at this stage by means of stained preparations shows, in fact, many pathological nuclei, and indications in some of them that chromatin material has been lost or thrown out. The possibility that parthenogenesis may rarely take place and account for some of the matroclinous plutei is admitted, but control experiments show that this must be a very rare occurrence, if it occurs at all, and will not suffice to explain the condition of the great majority of cases. It may rarely occur that the sperm-nucleus does not enter into combination with the egg-nucleus. Such eggs also might be expected to produce purely matroclinous plutei.

Echinus by *Strongylocentrotus.* When the eggs of Echinus are fertilized by sperm of Strongylocentrotus, hybrid plutei may be obtained, but since the two parental larval forms are closely

similar it is not possible to determine which parent the hybrid resembles more closely. The reciprocal cross is more difficult to make. This hybrid pluteus also is like that of the other combination. In each of these species, the haploid number of chromosomes is 18, hence there should be 36 chromosomes in the hybrid, but Baltzer records finding only 32 at the 4-cell stage. He observed in one 4-cell stage two chromosomes lagging in the equator of the mitotic figure, but whether they were lost, or not, was not determined. Baltzer concluded that all, or nearly all of the chromosomes are, in this cross, included in the nuclei, and this is borne out by the size of the nuclei in the pluteus.

In one other case there was evidence of elimination of chromosomes, namely, in the cross between Arbacia female and Echinus male. After elimination from 28 to 30 chromosomes are left. This means that from eight to ten chromosomes are lost. According to Vernon the hybrid plutei of this cross are of the pure Arbacia type.

Some of the material with which Shearer, de Morgan and Fuchs worked was turned over to Doncaster and Gray ('13) for cytological examination. It will be recalled that the breeding experiments in 1911 gave only matroclinous larvae, while those of 1912 gave evidence of paternal influence in some cases.

In the reciprocal cross the sperm-nucleus fuses with the egg-nucleus before it has enlarged. As the mitotic figure forms, 38 chromosomes can be counted. Abnormal features now appear; some of the chromosomes, or parts of them, swell into small vesicles. Their number varies from one to seven or even more. The chromosomes divide and pass to the poles, but those with the vesicles attached may not completely separate, and both halves may go to the same pole. Those vesicles that lie amongst the chromosomes may be carried to one or to the other pole; but those that lie at the periphery may be left at the equator and fail to be included in the daughter nuclei. At the second division the same state of affairs appears again. Vesicles appear, some of them go to the poles, others are left at the equator. Some of the vesicles appear to be pieces of chromosomes that have broken off.

When the eggs of E. acutus are fertilized by sperm of E. miliaris a few vesicles are sometimes found in the segmentation spindle. No trace of elimination was seen (see below), although

irregular distribution of a few chromosomes may possibly occur. E. acutus has 38 chromosomes and E. miliaris 34. The hybrid is expected to contain 36 chromosomes, and this number appears to be present.

In eggs procured in 1912 the results were somewhat different. When E. acutus eggs were fertilized by E. miliaris sperm no vesicles were found and no elimination observed. In the reciprocal cross few eggs were fertilized. Greater irregularity of the chromosomes in the segmentation spindle was observed. In the late phases of division two or sometimes more chromosomes lag on the spindle and may be excluded from the daughter nuclei.

How far these results will account for the facts observed and recorded by Shearer, de Morgan and Fuchs is uncertain. That elimination may occur, and then probable elimination of paternal chromosomes, might seem to account for the maternal inheritance, but the difference in the results in 1911 and 1912 cannot be explained clearly by the observed behavior of the chromosomes in the different years. It is significant that in the cross, E. esculentus female by E. miliaris male which gave maternal plutei in both years, no elimination of any kind was observed. This result seems to show that the matroclinous characters, in this cross at least, are not due to elimination, but possibly to the failure of the chromosomes of miliaris to produce an effect on the esculentus eggs.

Tennent ('12) has followed the chromosomes in a cross between two sea-urchins, Toxopneustes and Hipponoë. While there are constant differences in the shapes of the chromosomes of the two species, it was not found possible to distinguish the two kinds in the cross-fertilized eggs—with the exception of a hook-shaped chromosome. Eggs of Toxopneustes fertilized by sperm of Hipponoë show the double number of chromosomes in the segmentation nucleus (circa. 18 plus 16). The chromosomes divide regularly. In anaphases 30 to 34 chromosomes were present. The same number are found up to the 32- and 64-cell stages. There appears to be no elimination when the cross is made this way. The dominance of Hipponoë characters is not due, then, to elimination.

In the reciprocal cross, Hipponoë eggs by Toxopneustes sperm, the behavior of the chromosomes is irregular in the first cleavage spindle. In the anaphases of the first division from

22 to 32 chromosomes were counted; in the 4-cell stage 25 and 30; in the 16-cell stage 16 chromosomes were counted. It seems probable that there is an elimination, by which, in the extreme cases recorded in the 16-cell stage, the number is reduced to that of the egg. In these extreme cases it would appear that the "dominance" of Hipponoë is due to the presence of Hipponoë chromosomes alone. There is no inconsistency between the results in the two crosses, because it appears that when both are present even in the egg of Toxopneustes, the Hipponoë factors dominate; the loss of the Toxopneustes set in the reciprocal cross would, in any case, lead to the same result.

It may be recalled that Tennent found that when the alkalinity of the sea water was reduced (by acid), the Hipponoë dominance became less marked. A few observations on eggs of this kind (in the cross Toxopneustes female by Hipponoë male) lagging chromosomes were found. If these are Hipponoë chromosomes the decrease in "dominance" might be explained, provided the chromosomes that affect the hybrid pluteus characters are the ones lost. The evidence does not suffice to settle this point.

Tennent's results with pure species of Hipponoë show that an unpaired element (hook-shaped) is present in half of the eggs after fertilization. In the cross of Toxopneustes eggs by Hipponoë sperm, the hook-shaped chromosome is found in half of the eggs and is absent in the other half. In the reciprocal cross this hook-shaped chromosome is absent. It is evident that the hook-shaped chromosome is confined to the male line in Hipponoë, and that it must come only from the male as shown by the two crosses.

Tennent ('12) has examined the chromosome group of the sea-urchin Toxopneustes variegatus and of the spatangoid Moira atropos as well as that of the hybrid. Here the two kinds of chromosomes are so much alike that they cannot be distinguished in the hybrid.

These cytological studies of cross-fertilized eggs have given important information that bears directly on the interpretation of the characters of the hybrid plutei. There can be little doubt that Baltzer's interpretation is essentially correct. When elimination of some of the paternal chromosomes takes place it is a fair inference that the maternal character of the hybrid pluteus is due to this loss. This is especially clear in cases where, in the

reverse cross, there is no elimination and the hybrid pluteus is intermediate. The elimination in the later stages, blastula and gastrula, is no less significant, for, here too, the maternal character of the hybrid is probably due to loss of paternal chromosomes. This result suggests further that since the chromosomes may at first divide normally and only later become abnormal, there may be other cases when they may fail to function properly in late stages, even although they may not actually be eliminated or show visible pathological behavior.

Godlewski ('06) reported a cross between sea-urchin's eggs and crinoid sperm. The resulting plutei were purely matroclinous. The cross was made possible by the addition of a small amount of NaOH to the sea water in which the fertilization took place. Godlewski reports that no elimination of chromatin takes place during the early cleavages and that the size of the nuclei in the hybrid is intermediate between that of the nuclei of the parent types.

Baltzer ('10) has confirmed these conclusions and has added further details that make the results more probable. The eggs of Strongylocentrotus and of Echinus were fertilized by Antedon sperm. No evidence of elimination of chromosomes was found. The presence, in the first spindle, of some chromosomes that are not those of the sea-urchin is evidence that both sets take part in the cleavage. The number of chromosomes confirms this conclusion. Between 29 and 30 chromosomes are present in the early cleavage stages of the hybrid. If Strongylocentrotus (or Echinus) is responsible for 18 of them, then 11 or 12 are to be referred to Antedon, which about corresponds to the number ascribed by Godlewski to this crinoid. At the blastula stage the hybrid embryo begins to develop more slowly than the control (pure type). It may even come to a standstill. Sickly blastulae are often met with. If the embryo develops further it reaches the pluteus stage much later than does the control. The pluteus is strikingly of the maternal type, i.e., it possesses the echinoid skeleton that is lacking in the crinoid. No characteristics of the crinoid are present in the development or in the hybrid pluteus. The nuclei of the hybrid are approximately of the same size as those of the normal pluteus of Sphaerechinus or of Echinus. It seems then that no elimination has taken place in later stages.

In the cross it appears that although all the chromosomes

are present, only those derived from the egg have an influence on the character of the larvae. It is remarkable that the crinoid chromosomes are able to carry out their divisions in protoplasm of the sea-urchin's egg, especially when this does not occur between certain combinations of sea-urchins. It is less remarkable that, when present, the crinoid chromosomes fail to affect the character of the larvae.

Cross-Fertilization of Fishes

Teleostean fishes have furnished excellent material for experiments in cross-fertilization. Not only have closely related species been successfully crossed, but even species belonging to different genera, families, and sub-orders. No general rule can be stated, but, from closely related species, hybrid embryos that hatch and even reach maturity can sometimes be produced, while the more dissimilar the species the less likely are viable embryos to be expected. Their development may proceed in some cases only as far as the gastrula stage, while in other crosses abnormal embryos may develop which do not hatch.

Aside from these general considerations relating to fish hybrids there are certain more special problems which have been studied such as: the rates of cleavage and of development of the hybrid; the dominance of certain characters in the hybrids; the shape of the chromosomes in foreign cytoplasm; and the possibility of the elimination of some or all of the paternal chromosomes.

Amongst the earliest experiments were those of Appelöf ('94), who crossed such widely different species as the cod and the flounder, the cod and the cunner, the stickleback and the flounder, etc. He found that the cleavage was identical with that of the egg-species, although in rare cases its rate was retarded. He observed that the critical period in the development of the hybrid occurs at the end of the cleavage and the beginning of gastrulation—a conclusion confirmed by later observers.

In the same year ('94) Moenkhaus described crosses between Fundulus heteroclitus and Menidia notata. He found that the cleavage rate of the hybrid was the same as that of the egg-species, but that the embryos were deformed. At the time when the first segmentation spindle is present, the chromosomes derived

from the sperm-nucleus can be distinguished from those from the egg-nucleus; the former retaining their specific form in foreign cytoplasm. They retain their form even in later cleavages. No elimination of chromosomes was observed.

Newman ('08) made a detailed study of the hybrids of Fundulus heteroclitus and F. majalis. The eggs of majalis have twice the volume of those of heteroclitus, and develop more slowly. The rate of cleavage of both hybrid combinations is that of the egg-species. The earliest measurable effect of the foreign sperm is seen 14 to 20 hours after fertilization when the hybrid blastodisc (from heteroclitus female and majalis male) is slightly larger than the heteroclitus pure blastodisc. In the reciprocal cross, majalis female by heteroclitus male, the difference is not seen until four hours later. In general the development of the heteroclitus embryo is retarded by the introduction of majalis sperm, while the development of the majalis egg is accelerated by the heteroclitus sperm, but this difference is not permanent. "The more fortunate of the hybrids (from heteroclitus female by majalis male), although retarded for the first eight or ten days, are at and subsequent to hatching somewhat larger. They have a more rapid and efficient circulation, are more active in their movements, show greater resistance to lack of oxygen and the presence of carbon dioxide and live longer in captivity than do any of the pure heteroclitus embryos." On the other hand, while the hybrids from majalis female and heteroclitus male develop more rapidly than do pure majalis embryos for the first seven to ten days, later they cease to grow and "attain a size only half that of the pure majalis" embryos at the time of hatching, and never leave the egg-shell, apparently because of their inability to reduce the large amount of yolk.

The heart beat of the pure heteroclitus embryos appears about ten hours earlier than that of the hybrid (from heteroclitus female, by majalis male) and this gives the former an advantage in the subsequent rate of development, but when the heart beat of the hybrid overtakes that of the pure types (an endowment from the paternal species) the hybrid overhauls the pure species, and for a time is nearly on the same footing. The heart beat of the reciprocal hybrid (from majalis female by heteroclitus male), appears a day earlier than that of the pure majalis, which gives the hybrid for a time an advantage. But the pure form

soon attains a heart beat that is more rapid, so that it overtakes and passes the hybrid.

The appearance of the pigment seems parallel to that of the circulation and is dependent upon it. The pure heteroclitus embryos become heavily pigmented in about three days, while the majalis become pigmented very late in development and then lightly. The hybrid from heteroclitus female and majalis male develop pigment a day later than the pure heteroclitus embryos, and there is less of it, but in later stages the hybrid may surpass the pure form, because, apparently, the hybrid combines the heteroclitus character of densely packed chromatophores with the majalis character of darker pigment. The reciprocal hybrids (from majalis female by heteroclitus male) develop pigment earlier and more of it than the pure majalis. The color pattern on the head and trunk, characteristic of Fundulus heteroclitus embryos is found on all embryos of the hybrid from majalis female by heteroclitus male at the period of their maximum development. The heteroclitus type of chromatophores is found in both hybrid forms. On hatching, the majalis embryo is about twice the size of the heteroclitus embryos. The hybrid from heteroclitus female and majalis male is, at the time of hatching, no larger than the pure heteroclitus.

Moenkhaus ('10) described the results of thirty-four crosses between species of teleostean fishes, some of which were nearly "related," others belonged to different families or even to different orders. Cross-fertilization, followed by cleavage, took place in every case. The percentage of eggs that segmented was usually as high as 75 per cent or higher. Polyspermy was very exceptional. The proportion of eggs that segmented bore no close relation to the degree of relationship of the two species; but, on the other hand, the stage to which the embryos develop was found to be "correlated with the nearness of relationship of the two species used." The rate of development in the early cleavage is always that of the egg-species. "Any effect of the strange sperm upon the rate of development shows itself by slowing the process regardless of whether the rate of the sperm-species is faster or slower than that of the egg-species." The mortality of the hybrids is greatest at gastrulation. If the heart forms, even although it pumps no blood, the embryo may remain alive for some time.

Newman ('10) has compared the rate of cleavage of normal eggs of Fundulus majalis with that of the hybrid between F. majalis female and F. heteroclitus male. He concludes that the hybrid eggs cleave more rapidly than the eggs of the slower maternal species, due to the influence of the sperm of the more rapidly cleaving species. The effect may be produced by other elements than the chromosomes that are introduced by the sperm.

Bancroft ('12) crossed Fundulus heteroclitus and Fundulus majalis, the same species that Newman had studied, in order to examine more especially the inheritance of the pigment from a Mendelian point of view, i.e., the dominance or recessiveness of the pigment-bearing cells. He found that the large red chromatophores of heteroclitus are dominant over the few small yolk chromatophores of the majalis type. But as Newman had pointed out the dominant elements are fewer, smaller, and less branched than in heteroclitus. Bancroft found that the black yolk-chromatophores of heteroclitus are dominant over the same cells of majalis, but Newman thinks that they show in their size and shape some influence of the majalis species. Bancroft found "that the presence of a first crop of head chromatophores appearing before the majority of the head chromatophores (Fundulus heteroclitus condition) is dominant over the absence of this crop of head chromatophores (F. majalis condition)." Newman, on the other hand, states that he is unable to convince himself of the validity of the contrast, since he cannot discover any sharp demarkation for this group of chromatophores. He thinks that their time of appearance is strictly intermediate in the hybrid. Both authors show that the red chromatophores along the lateral line, at the time of hatching of the hybrid, are strictly dominant.

In addition to the above list, Newman adds two other dominant features. First the red chromatophores of the head (heteroclitus type) are dominant over their absence in the other type. Second, the fusion of the black chromatophores (heteroclitus type) is dominant over the absence of fusion (majalis type). "In the hybrids, however, fusion is never so complete as in the pure F. heteroclitus."

It may be recalled here what has already been said in connection with the same problem in hybrid echinoderms, namely, that whether a character is completely dominant or intermediate in the F_1 hybrid is not a criterion as to whether it is Mendelian.

An intermediate hybrid character may be just as good a Mendelian character as one that is completely dominant in the hybrid. And, as Newman points out, in fact, an F_2 generation would be necessary to determine whether segregation of these pairs of contrasted characters takes place.

In his paper of 1914, just referred to, Newman not only made use of the two species of Fundulus already mentioned, but of a third species also, viz., Fundulus diaphanus, as well as a fourth member of the same family, Cyprinodon variegatus. He studied in detail the rates of development of the different hybrids in comparison with the rates of the parent species, and found that while the rate of development of the hybrids is often intermediate, yet in some combinations the hybrids have a higher rate than that of either parent species, and in others a lower rate than either. He states that he is inclined to believe that it will be found generally true "that in crosses between very closely related species the rate of development will be accelerated, while in those between distantly related species development will be retarded, but not necessarily in proportion to the heterogeneity of the cross." He concludes, therefore, that although foreign sperm may materially alter the rate of early development, it plays no rôle in the heredity of the organism until embryonic differentiation is well under way. There is, however, nothing at all inconsistent in the speeding or delay of development of the hybrid with hereditary processes; for students of heredity are quite familiar with similar phenomena and consider them in no sense antagonistic to the ordinary interpretation of Mendelian behavior.

Loeb ('12) cross-fertilized the eggs of Fundulus heteroclitus with the sperm of Menidia, Ctenolabrus and Stenotomus and obtained embryos that lived for a month or longer. The embryos were abnormal in various ways. In the absence of traces of paternal characters he concluded that the embryos were not hybrid but maternal, because, as he inferred, the sperm chromosomes took no part in the development except to start it. No histological evidence was given in support of this conclusion and it is not in agreement with other evidence furnished by Moenkhaus, Morris, Pinney and G. and P. Hertwig. Moreover Newman, who has more recently gone over the same ground in much more detail finds clear evidence in some cases of the effects of the paternal chromosomes on the hybrids. In one case only (Fun-

dulus heteroclitus by Menidia) Loeb found evidence of paternal influence and this has also been confirmed by Newman. In addition, Newman describes pigment characters in the hybrids that must be ascribed to the father in the cross between Fundulus heteroclitus female by Scomber scombrus male, as well as in other cases that are described in his paper of 1915.

In the latter paper Newman describes 93 crosses among 14 species. Of these, 78 were between different genera (heterogenic). He finds little correlation between the success in development and the nearness of relationship of the species crossed. Other factors in the egg, not well made out, are possibly more important than "relationship." He also found that the eggs of some species never hybridize well, while the eggs of other species appear to have a high capacity "with almost any other species." For example, the eggs of Fundulus majalis develop poorly even when fertilized by closely related species of the same genus, for no hybrid larvae were ever seen to hatch and even the circulation fails to develop. Cyprinodon, Apeltes and Gasterosteus also cross badly even with their closest relatives. Some pelagic eggs such as those of the scup (Stenotomus) and cunner hybridize very successfully; but others such as those of the tautog and mackerel do so poorly. The three fish that hybridized with the greatest success were Tautogolabrus, Stenotomus, and Menidia. The eggs of Fundulus heteroclitus hybridize exceptionally well and often produce late larvae with a good circulation.

Newman states that "there are certain indications also that some species of sperm are better adapted to hybridization than others." The sperm of Fundulus heteroclitus, Menidia and Poronotus give a better set of hybrids than does that of Stenotomus, Cyprinodon, or of Tautoga, but on what the difference depends is not known.

It might be expected that species having the same developmental rate would give more harmonious results than when species with very different rates are crossed, but this turns out to be true only to a very limited extent. "The various species of pelagic eggs which hatch in two or three days unquestionably hybridize together with more general success than do these species when crossed with forms that have a slow developmental rhythm." But there are exceptions even to this rule; for Menidia eggs that require two weeks to develop, develop well with Poronotus

sperm whose embryos hatch in about two days. Again Fundulus heteroclitus, which hatches in two weeks, crosses successfully with Scomber scombrus that hatches in two or three days.

The Behavior of the Chromosomes in Hybrid Fish

The earlier work of Moenkhaus, and the later work of Morris, G. and P. Hertwig, and especially that of Pinney has shown that in hybrid fish the sperm-nucleus combines with the egg-nucleus as in normal fertilization, and that both sets of chromosomes are drawn into the first segmentation spindle. Little or no evidence of elimination of chromosomes has been found in the first division by any of these observers except Pinney, whose thorough and detailed account makes it quite clear that in several cases elimination of chromosomes takes place. That the abnormal development seen in many hybrids may be traced to this elimination is highly probable, but that all of the results are not due to this process is made equally clear. The following examples will give some further details.

When Ctenolabrus adspersus female was crossed by Stenotomus chrysops male many of the eggs developed normally up to the time of hatching, but none hatched. Newman had obtained, however, many hatching embryos of the maternal type from this cross. No abnormalities in the behavior of the chromosomes in the early cleavages were found, or even in the few cases of later cleavages that were examined.

The reciprocal cross, Stenotomus female by Ctenolabrus male, never passed beyond the gastrulation stages. In the first spindle two groups of chromosomes are found, maternal and paternal. In all the first three cleavage stages evidences of lagging chromosomes are present. In some cases undivided chromosomes may be observed passing to one pole; and evidence of breaking down of some chromosomes is everywhere apparent. Not all the chromatin reaches the poles of the spindles, and some of it is probably absorbed by the cytoplasm (Fig. 263*a*).

When Ctenolabrus female was crossed by Menidia male, stages later than gastrulation were not found by Newman; but Pinney found some abnormal embryos alive after three days. Two groups of chromosomes appear in the first spindles. No elimination at this time or later was discovered.

The reciprocal cross, Menidia female by Ctenolabrus male,

gave two larvae that hatched. Both were pure Menidia larvae. Evidences of lagging of chromosomes were found and abnormal mitosis was of frequent occurrence (Fig. 263*b*).

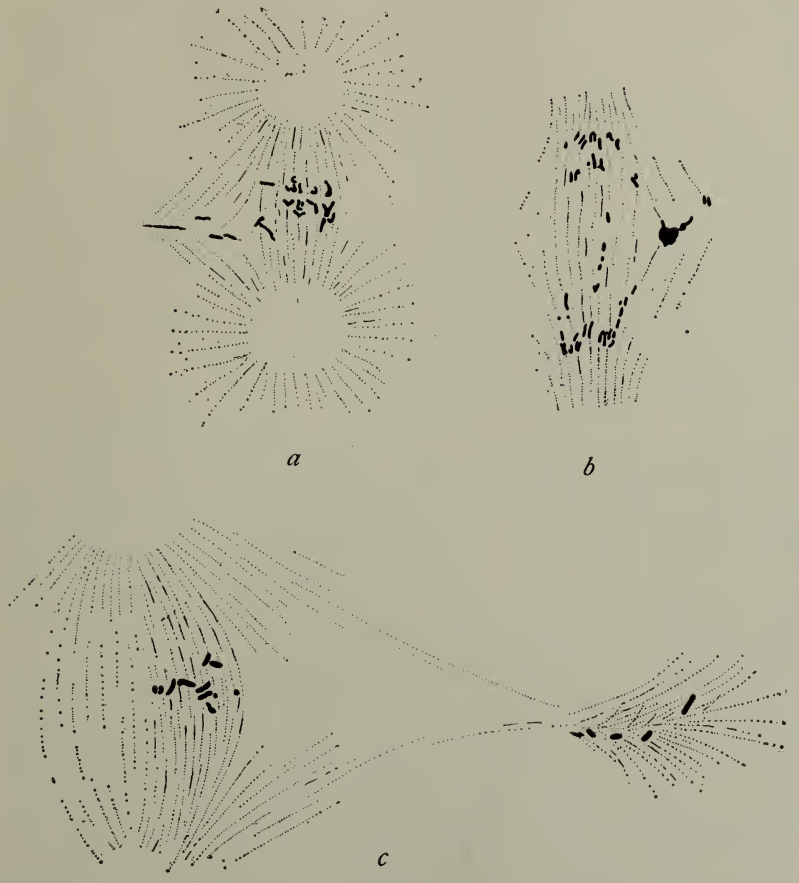


FIG. 263.—*a*, metaphase of second cleavage with elimination of chromosomes (to left) in hybrid between Stenotomus ♀ by Ctenolabrus ♂; *b*, anaphase of third cleavage, with chromosome clumps at equator in hybrid between Menidia ♀ by Ctenolabrus ♂; *c*, metaphase spindle of two-cell stage of hybrid (chromosome elimination in plane of first cleavage) between Fundulus ♀ by Ctenolabrus ♂. (After Pinney.)

When Ctenolabrus female was crossed by Fundulus male the development ceased at gastrulation. The earlier mitosis is prevailingly normal. Both kinds of chromosomes can be identified on the spindles.

The reciprocal cross, Fundulus female by Ctenolabrus male, gave many advanced embryos and one hatching embryo that was matroclinous. The first spindles did not show lagging chromosomes, but cases of displaced chromosomes were evident in the second spindles. These were chromosomes that had probably lagged in the first division. It is not clear, however, how far lagging and elimination are regular phenomena in this cross (Fig. 263*c*).

In conclusion, then, it seems probable that many of the abnormalities seen in the embryos may be due to chromosomal irregularities. There is, however, no evidence to show that all the paternal set is lost even if some of them are at times rejected. On the other hand, it is equally clear that in cases where normal mitosis followed by abnormal embryos was found, the latter cannot be ascribed to chromosomal irregularities, but rather to some sort of incompatibility between the effects produced by the two sets of chromosomes in the same cells.

Pinney's work draws attention to another important consideration. In those cases where the paternal chromosome belongs to a species whose segmentation is several hours later than that of the egg-species, the paternal set must be brought to the division phases much sooner than they would under normal conditions. Yet they do divide and in some cases apparently normally. We must suppose then either that they are ready for division long before they are divided under normal conditions, or else that their division is hastened in the speedier egg.

Since all authors agree that the tempo of the first divisions is that of the egg, it follows that even if a centrosome is brought in by the sperm as described by Whitman and Agassiz, the asters and the mitotic figure that are formed from the cytoplasm of the egg develop at the normal rate for the egg and not at that for the sperm-species. This suggests, at least, that the centrosome is not "specific," and such a view is in accordance with other results showing that the asters can be caused to develop by many kinds of external agents.

There is a further point suggested by Pinney's work. If the maternal set of chromosomes is divided normally in the hybrid egg, it is not evident why a normal embryo of pure maternal type might not be expected to develop, regardless of whether some or even all of the paternal set are lost. But in the first place there is evidence from other sources indicating that the haploid

set does not always suffice to give rise to a normal embryo; and in the second place, if only some of the paternal set are present (with the half maternal set) the balance may be upset, and irregularities result. Again there is the possibility that the fragmentation and vacuolation of paternal chromosomes may sometimes clog up the mechanism of mitosis and the maternal chromosomes may be irregularly distributed. While there is not much actual evidence that this occurs, nevertheless it may take place, at times, and be a further cause of abnormal development.

Several fish crosses have been made by G. and P. Hertwig ('14) the results of which do not differ from those already described. Gobius jozo female by Gobius capito male gave abnormal embryos; a few developed to a stage when their similarity to the embryos of Gobius jozo was apparent. Others showed evidence in the pigment of paternal influence. In the rate of development also they showed evidence of being intermediate. The reciprocal cross gave rise to hatching embryos. Evidence of paternal inheritance was found.

When Gobius jozo female was crossed to Crenilabrus pavo male only pathological embryos were obtained. Sections of the 2-cell stage showed normal mitotic figures. In the reciprocal cross the egg died before gastrulation, although 100 per cent of the eggs were fertilized.

Crosses between Crenilabrus pavo female and Box boops male gave embryos scarcely distinguishable from those of the mother-species, except that the pigment on the tail showed the influence of the sperm. Crenilabrus pavo female by Smaris alcedo male showed crenulation of the surface during the early cleavage, yet from the least modified eggs a few embryos developed.

The abnormalities in the development were not found to be connected with chromosome elimination, but the very limited number of observations made by the Hertwigs scarcely suffice to establish these conclusions. They ascribe the abnormalities to "idioplasmic disharmonies" between the maternal and paternal nuclear substances, which after all tells us no more than that the embryos from widely different forms develop abnormally.

Conclusions

That Mendel's theory should apply to embryonic larval characters, as well as to characters of adult animals, is, of course, entirely consistent with the fundamental principles of that theory. The demonstration of its application to larval stages is, however, possible only when contrasted pairs of characters are studied; for the alternative nature of the characters cannot be determined by inspection, or even in the first generation, but by their separation in the second and later generations. A modified form of Mendelian inheritance is met with when certain characters, relating to the egg and to the early stages of the embryo, are studied. The same principles apply strictly, but the result is masked, at first, because the cytoplasm of the egg has already developed under the influence of genes of the egg. Any opposing influence that the genes of the sperm have brought in have not, as yet, had time to produce this effect. That such genes of paternal origin can and do influence the cytoplasm of the egg is shown by later generations from such a cross. This kind of inheritance, generally called maternal inheritance, also occurs in species crosses, and can be recognized as such, even although the alternate or contrasted characters are unknown. If, for instance, the type of cleavage of the egg, or the method of gastrulation of the species to which the egg belongs, is different from the type of cleavage or gastrulation of the egg of the species to which the sperm belongs, these stages in the cross-fertilized egg may be like those typical of the maternal species. It has not been possible, as yet, to demonstrate that the explanation of this phenomenon in species-crosses is that indicated above, because later generations have not as yet been studied, but that the explanation is the one given cannot be doubted. The same phenomena appear in the silkworm's egg, where it has been shown that the maternal inheritance is strictly in accord with Mendelian principles.

There is another interest connected with matroclinous inheritance. It furnishes an opportunity of finding out the length of time required for the genes brought in by a sperm to produce an effect in the cytoplasm. As yet the evidence is rather meager, and largely comes from crosses of sea-urchins and from crosses of fish, but it suffices to show that there is a time element

involved. If this is granted, it seems probable that the genes affect the cytoplasm, not immediately in a dynamic sense, but rather by some material product that is set free from the genes into the cytoplasm. The evidence is not demonstrative, it must be conceded, but seems to be the simplest explanation of what has been found.

The evidence from observations on the cleavage of the cross-fertilized egg of sea-urchins and of fish goes to show that chromosomal elimination may take place in certain combinations, and there has been a tendency to interpret the maternal characters of the hybrid larvae as due to the loss of one or more of the chromosomes derived from the sperm of the other species. Here modern genetic work has contributed evidence that bears on certain implications in this interpretation. It has been shown, for instance, by Goodspeed and Clausen ('17), in a cross between two different species of tobacco, that the adult hybrid may show only the maternal character. Nevertheless, when the hybrid is back-crossed to the paternal species, there is evidence that the paternal chromosomes were present in the hybrid, but had produced no effect on its characters, which, as stated, are those of the maternal species. When these chromosomes are recovered by back-crossing, they show no evidence of having been changed in their sojourn in the cells of the hybrid. The simplest explanation appears to be that the foreign chromosomes have not been able to affect the cytoplasm derived from the other species in the presence of a single set of chromosomes of this species. Similar results may be expected to turn up at times in such widely different species-crosses as those of the sea-urchin and of fish. Should this happen the maternal character of the young hybrid might not be due to the elimination of paternal chromosomes. On the other hand, reciprocal crosses, whenever they give identical adult hybrids (as appears to be the rule), furnish evidence that the cytoplasm generally responds to the influence of the foreign sperm. Where elimination is known to occur when the cross is made one way and not when made reciprocally, and if the former hybrid is maternal, the result may be due partly to the elimination and partly to the failure of some or all of the paternal chromosomes to function in the presence of a full set of homologous maternal chromosomes.

There is still another line of evidence supplied by genetics

that bears on one of the problems discussed by embryologists. It is sometimes implied, when an irregular distribution of chromosomes takes place at the first division (due to polyspermy or to some other disturbance), that a cell getting one set of chromosomes may develop normally irrespective of the presence of one or more additional chromosomes. Genetic evidence has, on the contrary, shown that the presence of such additional chromosomes is not to be ignored. The question of balance now appears to be one of the important relations concerning the action of the genes on the cytoplasm.

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PHYSICAL AND CHEMICAL CHANGES IN THE EGG AFTER FERTILIZATION

CHAPTER IX

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CLEAVAGE AND THE MECHANISM OF CLEAVAGE

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THE LOCALIZATION OF THE MEDIAN PLANE

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LOCALIZATION BEFORE CLEAVAGE: THE DEVELOPMENT OF EGG-FRAGMENTS

CHAPTER XV

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THE DEVELOPMENT OF WHOLE AND PARTIAL EMBRYOS FROM ISOLATED BLASTOMERES

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CHAPTER XVIII

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ARTIFICIAL PARTHENOGENESIS

CHAPTER XXIII

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